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(71) Applicant (for all designated States except US): LILLY ICOS LLC [US/US]; 1209 Orange Street, Wilmington, DE 19801 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): ORME, Mark, W. [US/US]; 4235 Francis Avenue, #203, Seattle, WA 98103 (US). SAWYER, Jason, Scott [US/US]; 5718 North Winthrop Avenue, Indianapolis, IN 46220 (US). DAUGAN, Alain, Claude-Marie [FR/FR]; Centre de Recherche Glaxo Smith Kline Courtaboeuf Z.A. de Courtaboeuf, 25, avenue de Québec, F-91940 Les Ulis (FR). BROWN, Raymond, F. [US/US]; 10518 Greenway

Drive, Fishers, IN 46038 (US). SCHULTZE, Lisa, M. [US/US]; 16110 N.E. 175th Street, Woodinville, WA 98072 (US).

(74) Agent: NAPOLI, James, J.; Marshall, Gerstein & Borun, 6300 Sears Tower, 233 South Wacker Drive, Chicago, IL 60606 (US).

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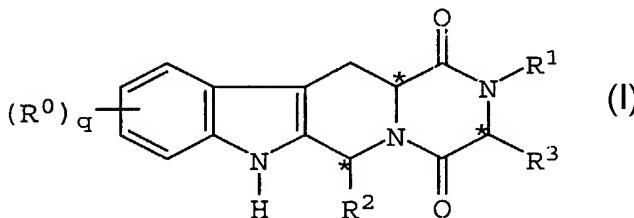
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(54) Title: CHEMICAL COMPOUNDS



(57) Abstract: Compounds of the general structural formula (I), and use of the compounds and salts and solvates thereof, as therapeutic agents.

WO 02/28858

CHEMICAL COMPOUNDS

CROSS REFERENCE TO RELATED APPLICATION

This application claims benefit of provisional U.S. patent application 60/237,477, filed October 2, 2000.

FIELD AND BACKGROUND OF THE INVENTION

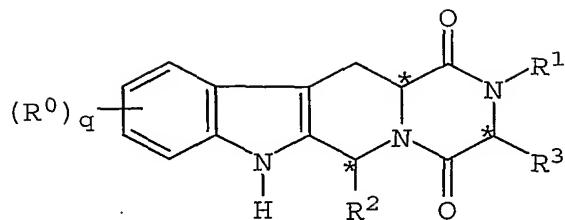
This invention relates to a series of compounds, to methods of preparing the compounds, to pharmaceutical compositions containing the compounds, and to their use as therapeutic agents. In particular, the invention relates to compounds that are potent and selective inhibitors of cyclic guanosine 3',5'-monophosphate specific phosphodiesterase (cGMP-specific PDE), in particular PDE5, and have utility in a variety of therapeutic areas wherein such inhibition is considered beneficial, including the treatment of cardiovascular disorders and erectile dysfunction.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compounds of formula (I)

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(I)

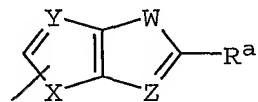
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wherein R⁰, independently, is selected from the group consisting of halo and C₁₋₆alkyl;

15 R¹ is selected from the group consisting of hydrogen, C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, haloC₁₋₆-alkyl, C₃₋₈cycloalkyl, C₃₋₈cycloalkylC₁₋₃alkyl, aryl-C₁₋₃alkyl, and heteroarylC₁₋₃alkyl;

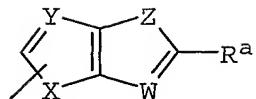
20 R² is selected from the group consisting of hydrogen, C₁₋₆alkyl, arylC₁₋₃alkyl, C₁₋₃alkenylaryl, haloC₁₋₆alkyl, C₁₋₄alkyleneC(=O)OR^a, C₁₋₄alkyleneC(=O)-NR^aR^b, C₃₋₈cycloalkyl, Het, C₃₋₈cycloalkenyl, C₃₋₈hetero-cycloalkenyl, C₁₋₄alkyleneHet, C₁₋₄alkyleneQR^a, C₂₋₆-alkenyleneQR^a, C₁₋₄alkyleneQC₁₋₄alkyleneQR^a,

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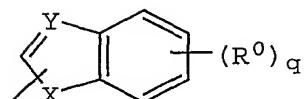


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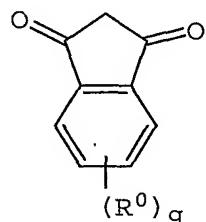
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and a spiro substituent having the structure

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R^3 is selected from the group consisting of hydrogen and C_{1-6} alkyl,

or R^1 and R^3 together represent a 3- or 4-membered alkyl or alkenyl chain component of a 5- or 25 6-membered ring;

R^a and R^b , independently, are selected from the group consisting of hydrogen, C_{1-6} alkyl, aryl, heteroaryl, aryl C_{1-3} alkyl, heteroaryl C_{1-3} alkyl, C_{1-3} -alkylenearyl, C_{1-3} alkyleneheteroaryl, and Het;

R^c is null or is selected from the group consisting of hydrogen, C_{1-6} alkyl, aryl, heteroaryl, aryl C_{1-3} alkyl, heteroaryl C_{1-3} alkyl, C_{1-3} alkylenearyl, C_{1-3} alkyleneheteroaryl, and Het;

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Het is a 5- or 6-membered heterocyclic group, saturated or partially unsaturated, containing at least one heteroatom selected from the group consisting of oxygen, nitrogen, and sulfur, and 5 optionally substituted with C_{1-4} alkyl or $C(=O)OR^a$;

Q is O, S, or NR^a ;

W is O, S, or NR^c ;

X is O, S, or NR^a ;

Y is CR^a or N;

10 Z is CR^a , $C(R^a)_2$, or NR^c ; and

q is 0, 1, 2, 3, or 4.

As used herein, the term "alkyl" includes straight chained and branched hydrocarbon groups containing the indicated number of carbon atoms, 15 typically methyl, ethyl, and straight chain and branched propyl and butyl groups. The term "alkyl" includes "bridged alkyl," i.e., a C_6-C_{16} bicyclic or polycyclic hydrocarbon group, for example, norbornyl, adamantyl, bicyclo[2.2.2]octyl, bicyclo[2.2.1]-heptyl, bicyclo[3.2.1]octyl, or decahydronaphthyl. 20 The term "cycloalkyl" is defined as a cyclic C_3-C_8 hydrocarbon group, cyclopropyl, cyclobutyl, cyclohexyl, and cyclopentyl. The terms "alkenyl" and "alkynyl" are defined similarly to the term "alkyl," 25 except the hydrocarbon group contains a carbon-carbon double bond or carbon-carbon triple bond, respectively. "Cycloalkenyl" is defined similarly to cycloalkyl, except a carbon-carbon double bond is present in the ring. The hydrocarbon group can 30 contain up to 16 carbon atoms.

The term "alkylene" refers to an alkyl group having a substituent. For example, the term " C_{1-3} alkylenearyl" refers to an alkyl group contain-

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ing one to three carbon atoms, and substituted with an aryl group. The term "alkenylene" as used herein is similarly defined, and contains the indicated number of carbon atoms and a carbon-carbon double bond, and includes straight chained and branched alkenylene groups, like ethylenylene.

The term "halo" or "halogen" is defined herein to include fluorine, bromine, chlorine, and iodine.

The term "haloalkyl" is defined herein as an alkyl group substituted with one or more halo substituents, either fluoro, chloro, bromo, iodo, or combinations thereof. Similarly, "halocycloalkyl" is defined as a cycloalkyl group having one or more halo substituents.

The term "aryl," alone or in combination, is defined herein as a monocyclic or polycyclic aromatic group, preferably a monocyclic or bicyclic aromatic group, e.g., phenyl or naphthyl, that can be unsubstituted or substituted, for example, with one or more, and in particular one to three, hydroxyalkyl, alkoxy, alkoxyalkyl, haloalkyl, nitro, amino, alkylamino, acylamino, alkylthio, alkylsulfinyl, and alkylsulfonyl. Exemplary aryl groups include phenyl, naphthyl, tetrahydronaphthyl, 2-chlorophenyl, 3-chlorophenyl, 4-chlorophenyl, 2-methylphenyl, 4-methoxyphenyl, 3-trifluoromethylphenyl, 4-nitrophenyl, and the like. The terms "aryl₁₋₃alkyl" and "heteroaryl₁₋₃alkyl" are defined as an aryl or heteroaryl group having a C₁₋₃alkyl substituent.

The term "heteroaryl" is defined herein as a monocyclic or bicyclic ring system containing one

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or two aromatic rings and containing at least one nitrogen, oxygen, or sulfur atom in an aromatic ring, and which can be unsubstituted or substituted, for example, with one or more, and in particular one to three, substituents, like halo, alkyl, hydroxy, hydroxyalkyl, alkoxy, alkoxyalkyl, haloalkyl, nitro, amino, alkylamino, acylamino, alkylthio, alkylsulfinyl, and alkylsulfonyl. Examples of heteroaryl groups include thienyl, furyl, pyridyl, oxazolyl, 10 quinolyl, isoquinolyl, indolyl, triazolyl, isothiazolyl, isoxazolyl, imidizolyl, benzothiazolyl, pyrazinyl, pyrimidinyl, thiazolyl, and thiadiazolyl.

The term "Het" includes a 5- or 6-membered heterocycloalkyl group exemplified by morpholinyl, 15 piperidyl, pyrrolidinyl, or piperazinyl.

The term "alkoxy" is defined as -OR, wherein R is alkyl.

The term "alkoxyalkyl" is defined as an alkyl group wherein a hydrogen has been replaced by 20 an alkoxy group. The term "(alkylthio)alkyl" is defined similarly, except a sulfur atom, rather than an oxygen atom, is present.

The term "hydroxy" is defined as -OH.

The term "hydroxyalkyl" is defined as a 25 hydroxy group appended to an alkyl group.

The term "amino" is defined as -NH₂, and the term "alkylamino" is defined as -NR₂, wherein at least one R is alkyl and the second R is alkyl or hydrogen.

The term "acylamino" is defined as 30 RC(=O)N, wherein R is alkyl or aryl.

The term "alkylthio" is defined as -SR, where R is alkyl.

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The term "alkylsulfinyl" is defined as R-SO₂, where R is alkyl.

The term "alkylsulfonyl" is defined as R-SO₃, where R is alkyl.

5 The term "nitro" is defined as -NO₂.

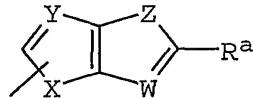
The term "trifluoromethyl" is defined as -CF₃.

The term "trifluoromethoxy" is defined as -OCF₃.

10 The term "cyano" is defined as -CN.

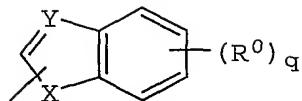
The term "spiro" as used herein refers to a group having two carbon atoms directly bonded to the carbon atom to which R² is attached.

15 In preferred embodiments, R² is optionally substituted and selected from the group consisting of C₁₋₄alkyleneQR^a, C₁₋₄alkyleneQC₁₋₄alkyleneQR^a, C₃₋₈-cycloalkyl, C₃₋₈cycloalkenyl, C₁₋₆alkyl,



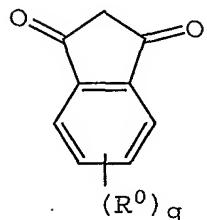
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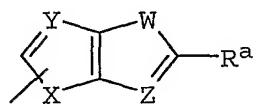
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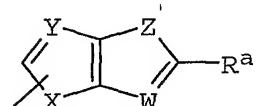
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In a more preferred group of compounds of formula (I), R^2 is represented by

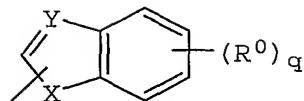


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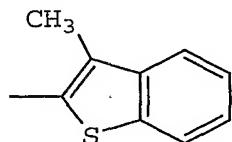
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C_{3-8} cycloalkyl, C_{3-8} cycloalkenyl, C_{1-6} alkyl, C_{1-4} alkyl-
eneQR^a, and C_{1-4} alkyleneQC₁₋₄alkyleneQR^a. A preferred
Q is oxygen.

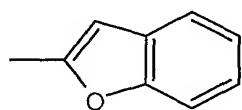
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Especially preferred R² substituents in-
clude

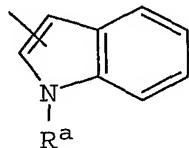
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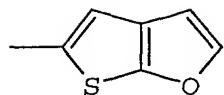


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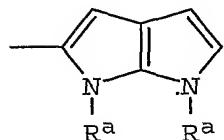


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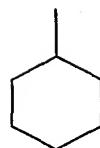
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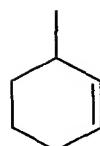
-CH₂OR^a, -CH₂OCH₂OR^a,

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, and

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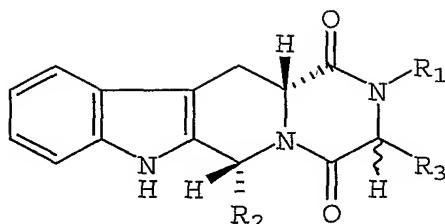
Within this particular group of compounds, preferred R^a substituents include hydrogen, C₁₋₆alkyl, and benzyl.

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An especially preferred subclass of compounds within the general scope of formula (I) is represented by compounds of formula (II)

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(II)

Compounds of formula (I) can contain one or more asymmetric center, and, therefore, can exist as stereoisomers. The present invention includes both mixtures and separate individual stereoisomers of the compounds of formula (I). Compounds of formula (I) also can exist in tautomeric forms, and the invention includes both mixtures and separate individual tautomers thereof.

Pharmaceutically acceptable salts of the compounds of formula (I) can be acid addition salts formed with pharmaceutically acceptable acids. Examples of suitable salts include, but are not limited to, the hydrochloride, hydrobromide, sulfate, bisulfate, phosphate, hydrogen phosphate, acetate, benzoate, succinate, fumarate, maleate, lactate, citrate, tartrate, gluconate, methanesulfonate, benzenesulfonate, and p-toluenesulfonate salts. The compounds of the formula (I) also can provide pharmaceutically acceptable metal salts, in particular alkali metal salts and alkaline earth metal salts, with bases. Examples include the sodium, potassium, magnesium, and calcium salts.

Compounds of the present invention are potent and selective inhibitors of cGMP-specific PDE5. Thus, compounds of formula (I) are of inter-

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est for use in therapy, specifically for the treatment of a variety of conditions where selective inhibition of PDE5 is considered to be beneficial.

5 Phosphodiesterases (PDEs) catalyze the hydrolysis of cyclic nucleotides, such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). The PDEs have been classified into at least seven isoenzyme families and are present in many tissues (J.A. Beavo, *Physiol. Rev.*, 75, 10 p. 725 (1995)).

PDE5 inhibition is a particularly attractive target. A potent and selective inhibitor of PDE5 provides vasodilating, relaxing, and diuretic effects, all of which are beneficial in the treatment of various disease states. Research in this area has led to several classes of inhibitors based on the cGMP basic structure (E. Sybertz et al., *Expert. Opin. Ther. Pat.*, 7, p. 631 (1997)).

20 The biochemical, physiological, and clinical effects of PDE5 inhibitors therefore suggest their utility in a variety of disease states in which modulation of smooth muscle, renal, hemostatic, inflammatory, and/or endocrine function is desirable. The compounds of formula (I), therefore, 25 have utility in the treatment of a number of disorders, including stable, unstable, and variant (Prinzmetal) angina, hypertension, pulmonary hypertension, congestive heart failure, acute respiratory distress syndrome, acute and chronic renal failure, 30 atherosclerosis, conditions of reduced blood vessel patency (e.g., postpercutaneous transluminal coronary or carotid angioplasty, or post-bypass surgery graft stenosis), peripheral vascular disease, vascu-

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5 lar disorders, such as Raynaud's disease, thrombo-
cythemia, inflammatory diseases, stroke, bronchitis,
chronic asthma, allergic asthma, allergic rhinitis,
glaucoma, osteoporosis, preterm labor, benign pros-
tatic hypertrophy, peptic ulcer, male erectile dys-
function, female sexual dysfunction, and diseases
characterized by disorders of gut motility (e.g.,
irritable bowel syndrome).

10 An especially important use is the treat-
ment of male erectile dysfunction, which is one form
of impotence and is a common medical problem. Impo-
tence can be defined as a lack of power, in the
male, to copulate, and can involve an inability to
achieve penile erection or ejaculation, or both.
15 The incidence of erectile dysfunction increases with
age, with about 50% of men over the age of 40 suf-
fering from some degree of erectile dysfunction.

20 In addition, a further important use is
the treatment of female arousal disorder. Female
arousal disorders are defined as a recurrent inabil-
ity to attain or maintain an adequate lubrication/-
swelling response of sexual excitement until comple-
tion of sexual activity. The arousal response con-
sists of vasocongestion in the pelvis, vaginal lu-
brication, and expansion and swelling of external
25 genitalia.

30 It is envisioned, therefore, that com-
pounds of formula (I) are useful in the treatment of
male erectile dysfunction and female arousal disor-
der. Thus, the present invention concerns the use
of compounds of formula (I), or a pharmaceutically
acceptable salt thereof, or a pharmaceutical compo-
sition containing either entity, for the manufacture

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of a medicament for the curative or prophylactic treatment of erectile dysfunction in a male animal and arousal disorder in a female animal, including humans.

5 The term "treatment" includes preventing, lowering, stopping, or reversing the progression or severity of the condition or symptoms being treated. As such, the term "treatment" includes both medical therapeutic and/or prophylactic administration, as
10 appropriate.

15 It also is understood that "a compound of formula (I)," or a physiologically acceptable salt or solvate thereof, can be administered as the neat compound, or as a pharmaceutical composition containing either entity.

20 Although the compounds of the invention are envisioned primarily for the treatment of sexual dysfunction in humans, such as male erectile dysfunction and female arousal disorder, they also can be used for the treatment of other disease states.

25 A further aspect of the present invention, therefore, is providing a compound of formula (I) for use in the treatment of stable, unstable, and variant (Prinzmetal) angina, hypertension, pulmonary hypertension, chronic obstructive pulmonary disease, congestive heart failure, acute respiratory distress syndrome, acute and chronic renal failure, atherosclerosis, conditions of reduced blood vessel patency (e.g., post-PTCA or post-bypass graft stenosis),
30 peripheral vascular disease, vascular disorders such as Raynaud's disease, thrombocythemia, inflammatory diseases, prophylaxis of myocardial infarction, prophylaxis of stroke, stroke, bronchitis, chronic

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asthma, allergic asthma, allergic rhinitis, glaucoma, osteoporosis, preterm labor, benign prostatic hypertrophy, male and female erectile dysfunction, or diseases characterized by disorders of gut motility (e.g., IBS).

According to another aspect of the present invention, there is provided the use of a compound of formula (I) for the manufacture of a medicament for the treatment of the above-noted conditions and disorders.

In a further aspect, the present invention provides a method of treating the above-noted conditions and disorders in a human or nonhuman animal body which comprises administering to said body a therapeutically effective amount of a compound of formula (I).

Compounds of the invention can be administered by any suitable route, for example by oral, buccal, inhalation, sublingual, rectal, vaginal, transurethral, nasal, topical, percutaneous, i.e., transdermal, or parenteral (including intravenous, intramuscular, subcutaneous, and intracoronary) administration. Parenteral administration can be accomplished using a needle and syringe, or using a high pressure technique, like POWDERJECT™.

Oral administration of a compound of the invention is the preferred route. Oral administration is the most convenient and avoids the disadvantages associated with other routes of administration. For patients suffering from a swallowing disorder or from impairment of drug absorption after oral administration, the drug can be administered parenterally, e.g., sublingually or buccally.

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Compounds and pharmaceutical compositions suitable for use in the present invention include those wherein the active ingredient is administered in an effective amount to achieve its intended purpose. More specifically, a "therapeutically effective amount" means an amount effective to prevent development of, or to alleviate the existing symptoms of, the subject being treated. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

A "therapeutically effective dose" refers to that amount of the compound that results in achieving the desired effect. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, which is expressed as the ratio between LD₅₀ and ED₅₀. Compounds which exhibit high therapeutic indices are preferred. The data obtained from such data can be used in formulating a dosage range for use in humans. The dosage of such compounds preferably lies within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed, and the route of administration utilized.

The exact formulation, route of administration, and dosage can be chosen by the individual

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physician in view of the patient's condition. Dosage amount and interval can be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the therapeutic effects.

5 The amount of composition administered is dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration, and the judgment of the prescribing physician.

10 Specifically, for administration to a human in the curative or prophylactic treatment of the conditions and disorders identified above, oral dosages of a compound of formula (I) generally are about 0.5 to about 1000 mg daily for an average 15 adult patient (70 kg). Thus, for a typical adult patient, individual tablets or capsules contain 0.2 to 500 mg of active compound, in a suitable pharmaceutically acceptable vehicle or carrier, for administration in single or multiple doses, once or several times per day. Dosages for intravenous, 20 buccal, or sublingual administration typically are 0.1 to 500 mg per single dose as required. In practice, the physician determines the actual dosing regimen which is most suitable for an individual 25 patient, and the dosage varies with the age, weight, and response of the particular patient. The above dosages are exemplary of the average case, but there can be individual instances in which higher or lower dosages are merited, and such are within the scope 30 of this invention.

For human use, a compound of the formula (I) can be administered alone, but generally is administered in admixture with a pharmaceutical car-

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rier selected with regard to the intended route of administration and standard pharmaceutical practice. Pharmaceutical compositions for use in accordance with the present invention thus can be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries that facilitate processing of compounds of formula (I) into preparations which can be used pharmaceutically.

These pharmaceutical compositions can be manufactured in a conventional manner, e.g., by conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. Proper formulation is dependent upon the route of administration chosen. When a therapeutically effective amount of a compound of the present invention is administered orally, the composition typically is in the form of a tablet, capsule, powder, solution, or elixir. When administered in tablet form, the composition can additionally contain a solid carrier, such as a gelatin or an adjuvant. The tablet, capsule, and powder contain about 5 to about 95% compound of the present invention, and preferably from about 25 to about 90% compound of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, or oils of animal or plant origin can be added. The liquid form of the composition can further contain physiological saline solution, dextrose or other saccharide solutions, or glycols. When administered in liquid form, the composition contains about 0.5 to about 90% by weight of a compound of the present invention.

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tion, and preferably about 1 to about 50% of a compound of the present invention.

When a therapeutically effective amount of a compound of the present invention is administered by intravenous, cutaneous, or subcutaneous injection, the composition is in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred composition for intravenous, cutaneous, or subcutaneous injection typically contains, in addition to a compound of the present invention, an isotonic vehicle.

For oral administration, the compounds can be formulated readily by combining a compound of formula (I) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the present compounds to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by adding a compound of formula (I) with a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include, for example, fillers and cellulose preparations. If desired, disintegrating agents can be added.

For administration by inhalation, compounds of the present invention are conveniently delivered in the form of an aerosol spray presenta-

- 20 -

tion from pressurized packs or a nebulizer, with the use of a suitable propellant. In the case of a pressurized aerosol, the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges, e.g., gelatin, for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, e.g., in ampules or in multidose containers, with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and can contain formulatory agents, such as suspending, stabilizing, and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils or synthetic fatty acid esters. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension. Optionally, the suspension also can contain suitable stabilizers or agents that increase the solubility of the compounds and allow for the preparation of highly concentrated solutions. Alternatively, a present composition can be in powder form for con-

- 21 -

stitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

Compounds of the present invention also can be formulated in rectal compositions, such as 5 suppositories or retention enemas, e.g., containing conventional suppository bases. In addition to the formulations described previously, the compounds also can be formulated as a depot preparation. Such long-acting formulations can be administered by 10 implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds can be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion 15 exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Many compounds of the present invention can be provided as salts with pharmaceutically compatible counterions. Such pharmaceutically acceptable 20 base addition salts are those salts that retain the biological effectiveness and properties of the free acids, and that are obtained by reaction with suitable inorganic or organic bases.

In particular, a compound of formula (I) 25 can be administered orally, buccally, or sublingually in the form of tablets containing excipients, such as starch or lactose, or in capsules or ovules, either alone or in admixture with excipients, or in the form of elixirs or suspensions containing flavoring or coloring agents. Such liquid preparations 30 can be prepared with pharmaceutically acceptable additives, such as suspending agents. A compound also can be injected parenterally, for example,

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intravenously, intramuscularly, subcutaneously, or intracoronarily. For parenteral administration, the compound is best used in the form of a sterile aqueous solution which can contain other substances, for 5 example, salts, or monosaccharides, such as mannitol or glucose, to make the solution isotonic with blood.

For veterinary use, a compound of formula (I) or a nontoxic salt thereof, is administered as a 10 suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can readily determine the dosing regimen and route of administration that is most appropriate for a particular animal.

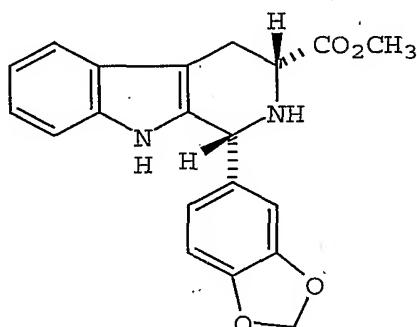
15 Thus, the invention provides in a further aspect a pharmaceutical composition comprising a compound of the formula (I), together with a pharmaceutically acceptable diluent or carrier therefor. There is further provided by the present invention a 20 process of preparing a pharmaceutical composition comprising a compound of formula (I), which process comprises mixing a compound of formula (I), together with a pharmaceutically acceptable diluent or carrier therefor.

25 In a particular embodiment, the invention includes a pharmaceutical composition for the curative or prophylactic treatment of erectile dysfunction in a male animal, or arousal disorder in a female animal, including humans, comprising a compound of formula (I) or a pharmaceutically acceptable 30 salt thereof, together with a pharmaceutically acceptable diluent or carrier.

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Compounds of formula (I) can be prepared by any suitable method known in the art, or by the following processes which form part of the present invention. In the methods below, R⁰, R¹, R², and R³ are as defined in structural formula (I) above. In particular, Daugan U.S. Patent No. 5,859,006, incorporated herein by reference, discloses preparation of a compound of structural formula (III).

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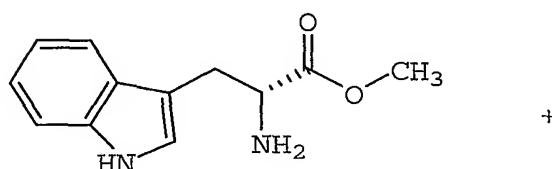
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(III)

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In short, the compound of structural formula (III), i.e., the *cis*-isomer of Intermediates 1 and 2 of Daugan U.S. Patent No. 5,859,006 was prepared according to the following reaction scheme:

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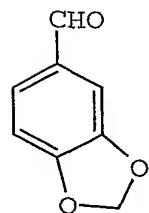


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D-Tryptophan methyl ester

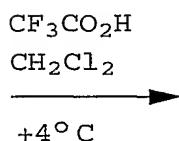
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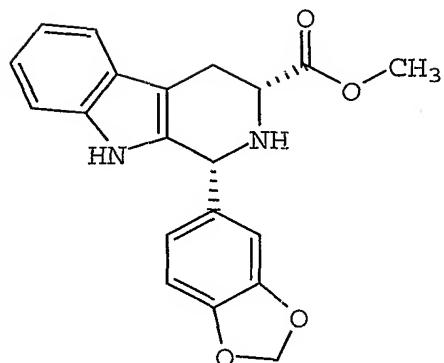
Piperonal

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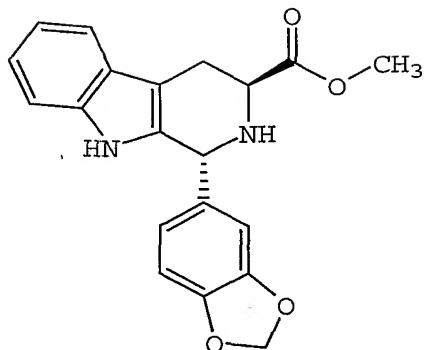
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(42%)

(III) (cis-isomer)

30

- 25 -



5

10

(28%)

(trans-isomer).

A compound of structural formula (I) is prepared similarly by reacting a tryptophan ester, 15 or a tryptophan ester substituted with suitable R^0 substituents, with a suitable aldehyde to provide the desired R^2 substituent. The resulting product then is cyclized by reaction with a suitable amine to provide a compound of structural formula (I).
20 The cyclization reaction is disclosed in Daugan U.S. Patent No. 5,859,006.

In the synthesis of compounds of structural formula (I), protecting compounds and protecting groups, like benzyl chloroformate and trichloroethyl chloroformate, which are well known to persons skilled in the art, can be used. Such protecting groups are disclosed, for example, in T.W. Greene et al. "Protective Groups in Organic Synthesis, Third Edition," John Wiley and Sons, Inc., NY, NY (1999).
25 The structure of a compound of structural formula (I) can be varied by using an appropriate aldehyde to change the identity of R^2 , or by using a halo or alkyl phenyl-substituted tryptophan ester.
30

- 26 -

Compounds of formula (I) can be converted to other compounds of formula (I). Thus, for example, when a compound contains a substituted aromatic ring, it is possible to prepare another suitably substituted compound of formula (I). Examples of appropriate interconversions include, but are not limited to, OR^a to hydroxy by suitable means (e.g., using an agent such as SnCl₂ or a palladium catalyst, such as palladium-on-carbon), or amino to substituted amino, such as alkylamine, using standard acylating or sulfonylating conditions.

Compounds of formula (I) can be prepared by the method above as individual stereoisomers from the appropriate stereoisomer of formula (III) or as a racemic mixture from the appropriate racemic compound of formula (III). Individual stereoisomers of the compounds of the invention can be prepared from racemates by resolution using methods known in the art for the separation of racemic mixtures into their constituent stereoisomers, for example, using HPLC on a chiral column, such as Hypersil naphthyl urea, or using separation of salts of stereoisomers. Compounds of the invention can be isolated in association with solvent molecules by crystallization from, or evaporation of, an appropriate solvent.

The pharmaceutically acceptable acid addition salts of the compounds of formula (I) that contain a basic center can be prepared in a conventional manner. For example, a solution of the free base can be treated with a suitable acid, either neat or in a suitable solution, and the resulting salt isolated either by filtration or by evaporation under vacuum of the reaction solvent. Pharmaceutically

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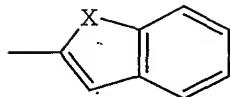
acceptable base addition salts can be obtained in an analogous manner by treating a solution of a compound of formula (I) with a suitable base. Both types of salt can be formed or interconverted using 5 ion-exchange resin techniques. Thus, according to a further aspect of the invention, a method for preparing a compound of formula (I), or a salt or solvate (e.g., hydrate) is provided.

The following abbreviations are used here-
10 after in the accompanying examples: rt (room tem-
perature), min (minute), h (hour), g (gram), mmol
(millimole), m.p. (melting point), eq (equivalents),
L (liter), mL (milliliter), μ L (microliters), DMSO
(dimethyl sulfoxide), CH_2Cl_2 (dichloromethane), CHCl_3 ,
15 (chloroform), TFA (trifluoroacetic acid), EtOH (eth-
anol), MeOH (methanol), Et₃N (triethylamine), MeNH₂
(methylamine), DMF (dimethylformamide), EtOAc (ethyl
acetate), S₈ (sulfur), DIPEA (diisopropylethylamine),
MOM-Cl (chloromethyl methyl ether), TSOH (p-toluene-
20 sulfonic acid), NaHCO₃ (sodium bicarbonate), Na₂SO₄
(sodium sulfate), AcOH (acetic acid), HCl (hydro-
chloric acid), LiAH₄ (lithium aluminum hydride), Et₂O
(diethyl ether), PCC (pyridinium chlorochromate),
Al₂O₃ (aluminum oxide), i-PrOH (isopropyl alcohol),
25 and THF (tetrahydrofuran).

General Synthesis of a Compound of Formula (I)

The following sequence illustrates a gen-
30 eral route to compounds of structural formula (I)
wherein R² is

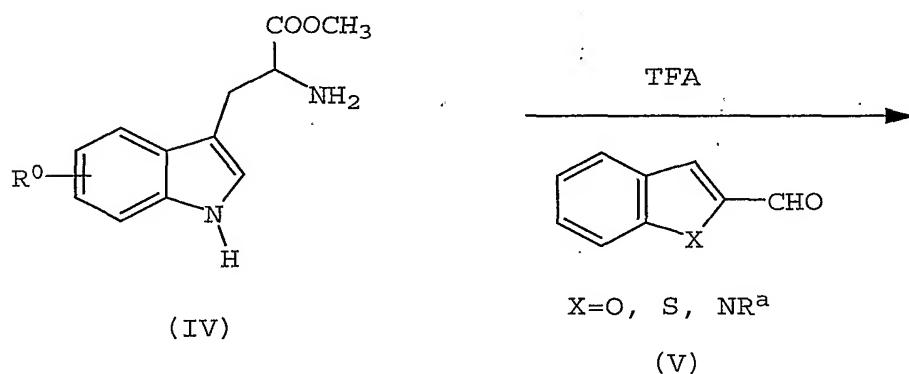
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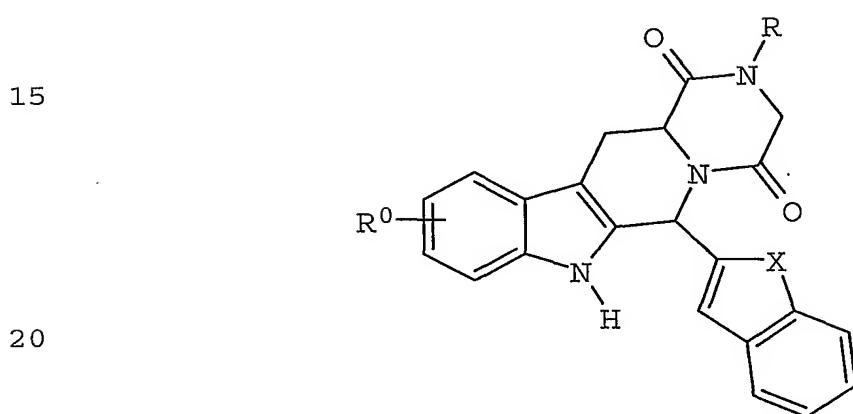
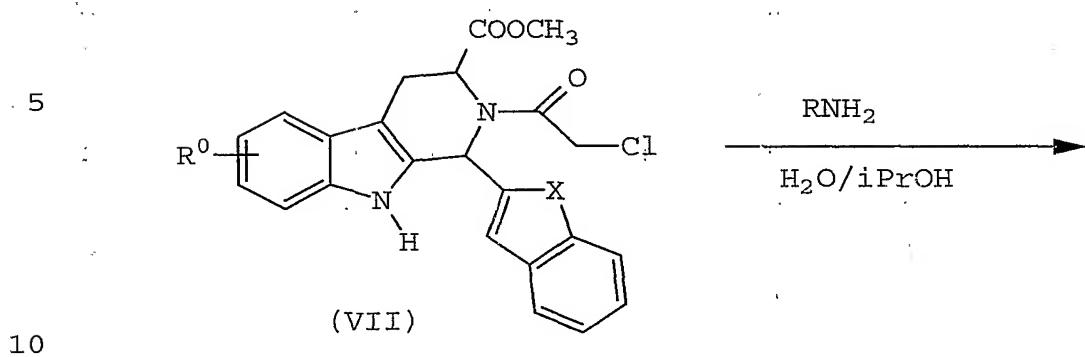
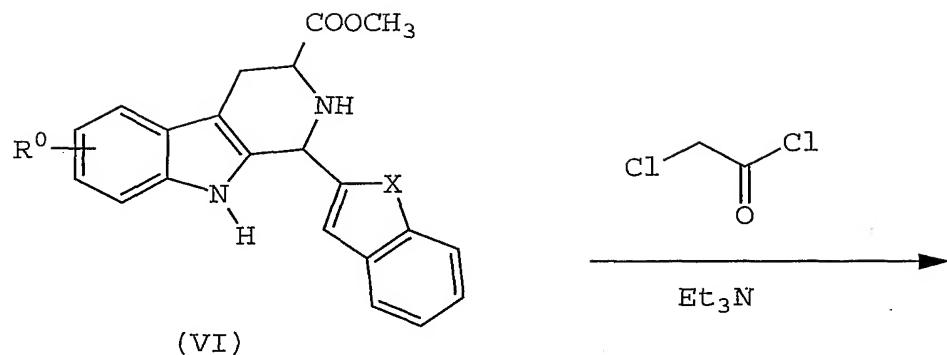
Similar synthetic routes can be used to synthesize other compounds of structural formula (I).

The general synthetic route is analogous to the route disclosed in U.S. Patent No. 5,859,006, incorporated by reference. In particular, a tryptophan ester (IV) is subjected to a Pictet-Spengler reaction with an appropriate aldehyde, such as (V) for example, in the presence of trifluorocetic acid (TFA) to provide a β -carboline (VI). Acylation with chloroacetyl chloride provides an N-derivatized compound (VII), which in turn is treated a desired primary amine (RNH_2) to provide a diketopiperazine compound (VIII) of structural formula (I).



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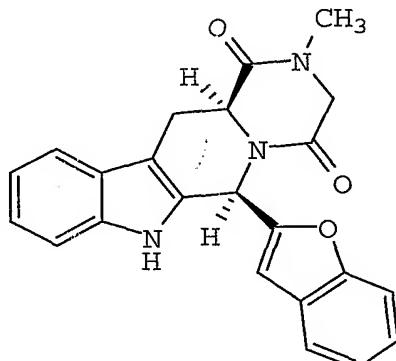
- 30 -

The following illustrates specific examples of compounds of structural formula (I) and synthetic routes to some of these structures.

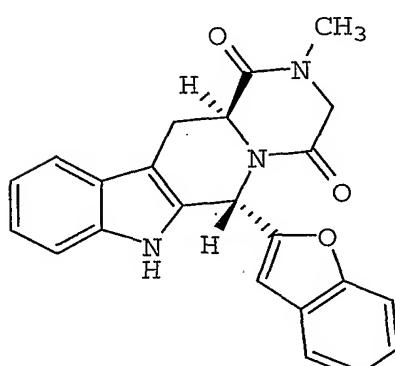
5 Preparation of Examples 1-14

The following Examples 1-14 were prepared by the above-described general synthetic scheme. The details of each step in the above synthetic 10 scheme are disclosed in U.S. Patent No. 5,849,006.

Preparation of Example 1



Example 1

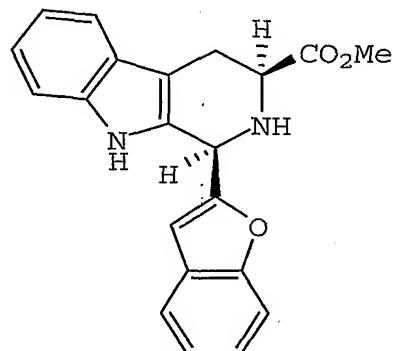


Example 2

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Example 1 was prepared from the following Intermediate 1. Example 2 was prepared from the following Intermediate 2.

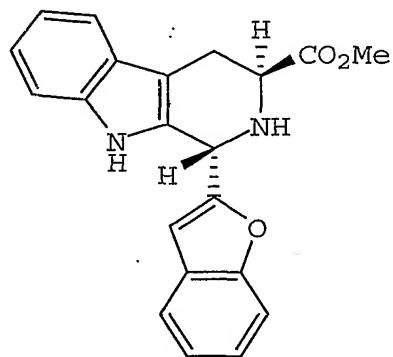
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Intermediate 1

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Intermediate 2

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Preparation of (1R,3S)- and (1S,3S)-1-benzofuran-2-yl-2,3,4,9-tetrahydro-1H- β -carboline-3-carboxylic acid methyl ester

(Intermediates 1 and 2)

5

Using benzofuran-2-carbaldehyde as the starting aldehyde, Intermediates 1 and 2 were prepared by the same method described below for (1R,3S)- and (1S,3S)-1-(3-methyl-benzo[b]thiophen-2-yl)-2,3,4,9-tetrahydro-1H- β -carboline-3-carboxylic acid methyl ester (Intermediates 3 and 4). The crude mixture of diasteromers was separated by chromatography (Chiralcel OD-H column, 60% heptane, 40% ethanol) to yield 2.5 g (56%) of (1R,3S)-1-benzofuran-2-yl-2,3,4,9-tetrahydro-1H- β -carboline-3-carboxylic acid methyl ester (Intermediate 1) and 920 mg (20%) of (1S,3S)-1-benzofuran-2-yl-2,3,4,9-tetrahydro-1H- β -carboline-3-carboxylic acid methyl ester (Intermediate 2) as foams.

(1R,3S)-1-Benzofuran-2-yl-2,3,4,9-tetrahydro-1H- β -carboline-3-carboxylic acid methyl ester (Intermediate 1): ^1H NMR (DMSO-d₆): δ 10.9 (s, 1H), 7.6-6.95 (m, 8H), 6.56 (s, 1H), 5.52 (d, J=4 Hz, 1H), 3.93 (m, 1H), 3.7 (s, 3H), 3.06 (dd, J=5.15 Hz, 1H), 2.8 (dd, J=10.15 Hz, 1H); MS ES+m/e 347.2 (p+1), ES-m/e 345.2 (p-1).

(1S,3S)-1-Benzofuran-2-yl-2,3,4,9-tetrahydro-1H- β -carboline-3-carboxylic acid methyl ester (Intermediate 2). ^1H NMR (DMSO-d₆): δ 10.66 (s, 1H), 7.7-6.95 (m, 8H), 6.9 (s, 1H), 5.55 (d, J=6 Hz, 1H), 3.9 (m, 1H), 3.64 (s, 3H), 3.05 (m, 1H), 2.85 (dd, J=2.10 Hz, 1H); MS ES+m/e 347.2 (p+1), ES-m/e 345.2 (p-1).

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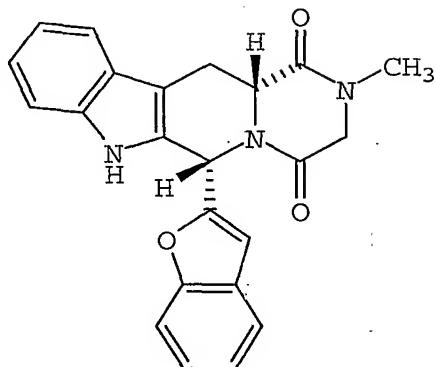
**Preparation of (6R,12aS)-6-benzofuran-2-yl-2-methyl-2,3,6,7,12,12a-hexahydro-pyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione
(Example 1)**

5

Example 1 was prepared from Intermediate 1 (2.66 mmol), triethylamine (1 mL), and chloroacetyl chloride (2.7 mmol) in tetrahydrofuran (25 mL). The mixture was stirred for 18 hours without cooling. The mixture then was diluted with ethyl acetate, washed once with water, once with 1 N hydrochloric acid, once with saturated sodium chloride solution, then dried (sodium sulfate), filtered, and concentrated *in vacuo*. The crude material was purified by chromatography (silica gel, 50% ethyl acetate, 50% hexanes, then ethyl acetate) to provide 852 mg (30%) of Example 1 as a solid: mp 195-198°C. ¹H NMR (DMSO-d₆): δ 11.6 (s, 1H), 7.65-7.0 (m, 9H), 4.4 (dd, J=4.13 Hz, 1H), 4.2 (ABq, J=18.65 Hz), 3.32 (dd, J=4.13 Hz, 1H), 3.05 (dd, J=13.5 Hz, 1H), 2.82 (s, 3H), MS ES+m/e 386.3 (p+1), ES-m/e 384.3 (p-1); IR (KBr, cm⁻¹) 1729, 1663, 1462.

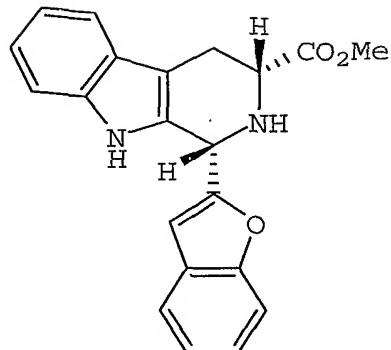
Example 2 was prepared by the same method as Example 1 from Intermediate 2.

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Preparation of Example 3

Example 3, (6S,12aR)-6-benzofuran-2-yl-2-methyl-2,3,6,7,12,12a-hexahdropyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione, was prepared from Intermediate 3 by the same method set forth above for Example 1. The crude material was purified by chromatography (silica gel, 30% ethyl acetate, 70% hexanes) to provide 540 mg (52%) of Example 3 as an off-white solid: mp 265-268°C. ^1H NMR (DMSO- d_6): δ 11.23 (s, 1H), 7.54 (m, 2H), 7.44 (d, $J=8$ Hz, 1H), 7.33 (d, $J=8$ Hz, 1H), 7.18 (m, 2H), 7.05 (m, 2H), 6.73 (s, 1H), 6.51 (s, 1H), 4.39 (dd, $J=4.11$ Hz, 1H), 4.26 (d, $J=17$ Hz, 1H), 3.98 (d, $J=17$ Hz, 1H), 3.59 (dd, $J=4.15$ Hz, 1H), 3.02 (m, 4H). MS ES+ m/e=386 (p+1). Anal. Calcd. for $\text{C}_{23}\text{H}_{19}\text{N}_3\text{O}_3$: C, 71.67; H, 4.96; N, 10.90. Found: C, 71.61; H, 4.82; N, 10.72.

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Intermediate 3

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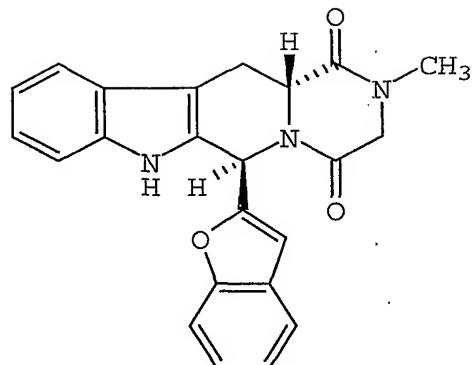
benzofuran-2-yl-2,3,4,9-tetrahydro-1H- β -carboline-3-carboxylic acid methyl ester (Intermediates 3 and 4). Condensation of (R)-(+)-tryptophan with benzofuran-2-carbaldehyde produced Intermediates 3 and 4 by the same method described above for Intermediates 1 and 2. The crude mixture of diastereomers was separated by chromatography (Biotage 75M, 20% ethyl acetate/80% hexanes) to give 2.85 g (44%) of (1R,3R)-1-benzofuran-2-yl-2,3,4,9-tetrahydro-1H- β -carboline-3-carboxylic acid methyl ester (Intermediate 4) and 950 mg (15%) of (1S,3R)-1-benzofuran-2-yl-2,3,4,9-tetrahydro-1H- β -carboline-3-carboxylic acid methyl ester (Intermediate 3) as foams.

(1S,3R)-1-Benzofuran-2-yl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (Intermediate 3): ^1H NMR (DMSO- d_6): δ 10.66 (s, 1H), 7.64 (m, 1H), 7.25 (m, 3H), 7.02 (m, 2H), 6.88 (s, 1H), 5.55 (s, 1H), 3.93 (m, 1H), 3.67 (s, 3H), 3.03 (m, 1H), 2.82 (m, 1H); TOF MS ES+ exact mass calculated for $\text{C}_{21}\text{H}_{19}\text{N}_2\text{O}_3$ (p+1): m/z -347.1396. Found: 347.1369.

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Example 4

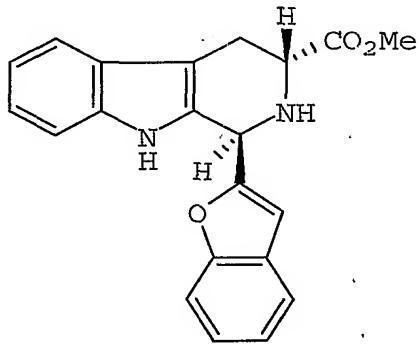
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Example 4 was prepared in an identical
15 manner as Example 3 from Intermediate 4.

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Intermediate 4

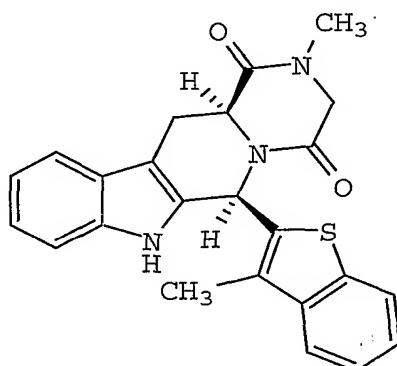
(1R,3R)-1-Benzofuran-2-yl-2,3,4,9-tetra-
hydro-1H-β-carboline-3-carboxylic acid methyl ester
30 (Intermediate 4): ^1H NMR (DMSO- d_6): δ 10.87 (s, 1H), 7.55 (s, 1H, $J=7$ Hz), 7.47 (d, 1H, $J=7$ Hz), 7.26 (m, 3H), 7.01 (m, 2H), 6.56 (s, 1H), 5.43 (s, 1H), 3.90 (m, 1H), 3.66 (s, 3H), 3.07 (dd, $J=4.15$

- 37 -

Hz, 1H), 2.80 (m, 1H); TOF MS ES+ exact mass calculated for $C_{20}H_{19}N_2O_3$ (p+1): m/z=347.1382. Found: 347.1382.

5 Preparation of Examples 5 and 6

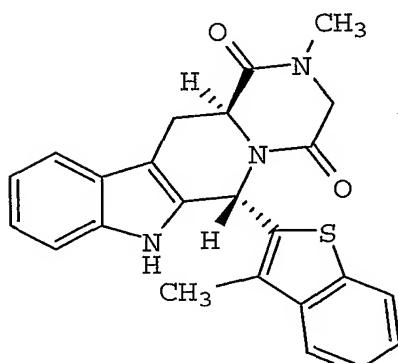
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Example 5

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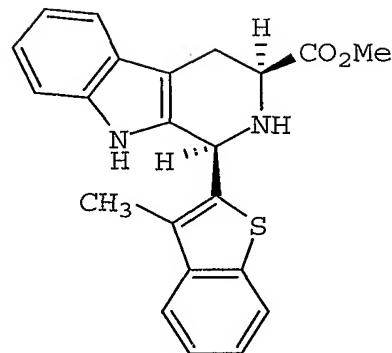
Example 6

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Example 5 was prepared from the following Intermediate 5. Example 6 was prepared from the following Intermediate 6.

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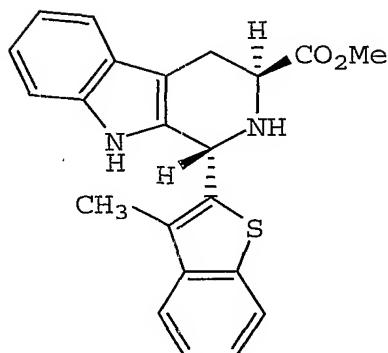
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Intermediate 5

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Intermediate 6

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Preparation of (1R,3S)- and (1S,3S)-1-(3-methylbenzo [b] thiophen-2-yl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester
(Intermediates 5 and 6)

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An aqueous solution of (S)-(-)-tryptophan hydrochloride (1.45 g, 5.7 mmol) was made alkaline with a saturated sodium bicarbonate solution and extracted with ethyl acetate. The extract was

- 39 -

washed once with aqueous sodium chloride solution, dried (sodium sulfate), filtered, and concentrated *in vacuo*. The free base was dissolved in chloroform (50 mL) and treated with 3-methylbenzo[b]thiophene-2-carbaldehyde (1.0 g, 5.7 mmol) and trifluoroacetic acid (21.2 mmol, 1.32 g). The resulting mixture then was heated to reflux for 3 hours. The solution was cooled to room temperature, washed once with saturated sodium bicarbonate solution, dried (sodium sulfate), filtered, and concentrated *in vacuo* to provide a mixture of two diastereomers. The isomers were separated by chromatography (silica gel, 30% ethyl acetate:hexanes) to give 700 mg (38%) of (1*R*,3*S*)-1-(3-methylbenzo[b]thiophen-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (Intermediate 5) and 400 mg (19%) of (1*S*,3*S*)-1-(3-methylbenzo[b]thiophen-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (Intermediate 6).

Intermediate 5: ^1H NMR (DMSO- d_6): δ 10.04 (s, 1H), 7.8-6.9 (m, 8H), 5.92 (d, $J=2$ Hz, 1H), 4.1 (dd, $J=5.10$ Hz, 1H), 3.65 (s, 3H), 3.45 (t, $J=2$ Hz, 1H), 3.03 (dq, $J=5.15$ Hz, 2H), 2.5 (s, 3H); MS ES+ m/e 377.1 (p+1), ES- m/e 375.2 (p-1).

(1*S*,3*S*)-1-(3-Methylbenzo[b]thiophen-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester (Intermediate 6): ^1H NMR (DMSO- d_6): δ 10.4 (s, 1H), 7.9-6.9 (m, 8H), 5.85 (br s, 1H), 4.0 (m, 1H), 3.75 (s, 3H), 3.0 (br s, 1H), 2.95 (dd, $J=6.10$ Hz, 2H), 2.5 (s, 3H); ME ES+ m/e 377.1 (p+1), ES- m/e 375.2 (p-1).

Example 5, (6*R*,12*aS*)-2-methyl-6-(e-methylbenzo[b]thiophen-2-yl)-2,3,6,7,12,12*a*-hexahydro-

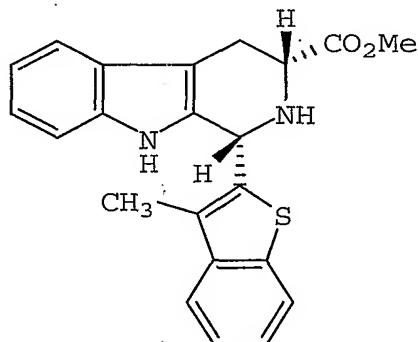
- 40 -

pyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione, was prepared by the same method given above for Example 1. The residue was dissolved in tetrahydrofuran (20 mL) and water (5 mL). A solution of 5 2M methylamine in tetrahydrofuran (5 mL, 20 mmol) was added and the reaction was heated at reflux for 30 minutes. After cooling to room temperature, the solvent was evaporated and the residue was diluted with ethyl acetate. A crystalline solid formed and 10 was filtered to give 220 mg (20%) of Example 3 as a colorless solid: mp 230-234°C. ¹H NMR (DMSO-d₆): δ 11.07 (s, 1H), 7.9-7.0 (m, 9H), 4.5 (dd, J=4.13 Hz, 1H), 4.2 (ABq, J=18.63 Hz), 3.35 (dd, J=4.13 Hz, 1H), 3.06 (dd, J=13.4 Hz, 1H), 2.82 (s, 3H), 2.56 15 (s, 3H); MS ES+m/e 416.1 (p+1), ES-m/e 414.2 (p-1); IR (KBr, cm⁻¹) 1728, 1677, 1649.

Example 6 was prepared in a manner identical to Example 5 from Intermediate 6.

Examples 7 and 8 are prepared in a manner 20 identical to Examples 5 and 6 from Intermediates 7 and 8.

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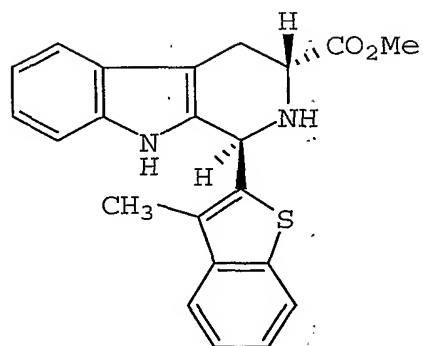


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Intermediate 7

- 41 -

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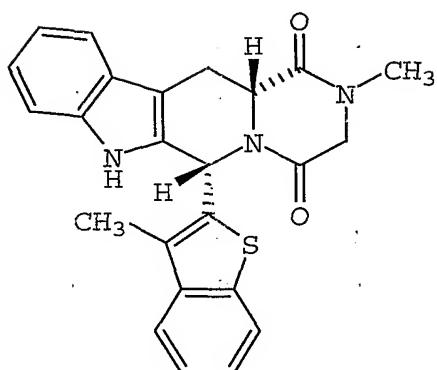


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Example 7

Intermediate 8

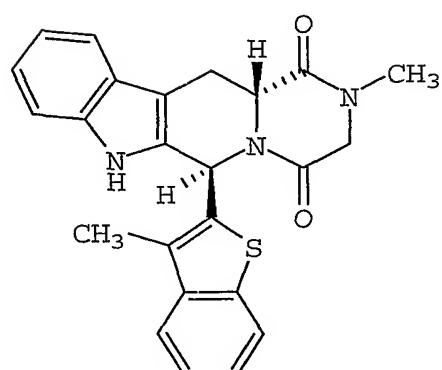
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Example 8

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Preparation of (1S,3R)- and (1R,3R)-1-(3-Methylbenzo[b]thiophen-2-yl)-2,3,4,9-tetrahydro-1H- β -carboline-3-carboxylic acid methyl ester
(Intermediates 7 and 8)

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An aqueous solution of (R)-(+)-tryptophan methyl ester hydrochloride (5.5 g, 19.6 mmol) was made basic with saturated sodium bicarbonate solution and extracted with ethyl acetate. The extract was washed with water, dried (magnesium sulfate), filtered, and concentrated *in vacuo* to produce a yellow oil (3.72 g). The free base was dissolved in chloroform (75 mL) followed by addition of 3-methylbenzo[b]thiophene-2-carboxaldehyde (3.0 g, 17.0 mmol) and trifluoroacetic acid (3.0 mL). The solution was stirred at 25°C for 14 hours, and at reflux for 1 hour. The solution was cooled and diluted with ethyl acetate (200 mL). The solution was extracted three times with ethyl acetate (100 mL), then the combined extracts were dried (magnesium sulfate), filtered, and concentrated *in vacuo* to give two diastereomers. The isomers were separated by chromatography (silica gel, 20% ethyl acetate/80% hexanes) to give 1.92 g (30%) of (1S,3R)-1-(3-methylbenzo[b]thiophen-2-yl)-2,3,4,9-tetrahydro-1H- β -carboline-3-carboxylic acid methyl ester and 1.68 g (27%) of (1R,3R)-1-(3-methylbenzo[b]thiophen-2-yl)-2,3,4,9-tetrahydro-1H- β -carboline-3-carboxylic acid methyl ester.

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Intermediate 7:

¹H NMR (DMSO-d₆) δ : 10.51 (s, 1H), 7.82 (t, J=6.22 Hz, 2H), 7.37 (m, 3H), 7.21 (d, J=6.95 Hz, 1H), 6.99

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- 43 -

(q, $J=8.14$ Hz, 2H), 5.90 (s, 1H), 3.98 (m, 1H), 3.75 (s, 3H), 3.20 (m, 1H), 3.06 (m, 1H), 2.84 (m, 1H), 2.53 (s, 3H); MS ES+ m/e 377.1 (p+1). Anal. Calcd. for $C_{22}H_{19}N_2O_2S$: C, 70.18; H 5.35; N 7.44.

5

Intermediate 8:

1H NMR (DMSO- d_6) δ : 10.54 (s, 1H), 7.81 (d, $J=8$ Hz, 1H), 7.32 (m, 3H), 7.20 (d, $J=7.6$ Hz, 1H), 7.01 (m, 2H), 5.93 (s, 1H), 4.10 (m, 1H), 3.64 (s, 3H), 3.43 (m, 1H), 3.07 (dd, $J=4, 15$ Hz, 2H), 2.51 (s, 3H); MS ES+ m/e 377.1 (p+1). Anal. Calcd. for $C_{22}H_{19}N_2O_2S$: C, 70.18; H 5.35; N 7.44. Found: C, 69.93; H, 5.31; N 7.33.

15

Preparation of Example 7

(6R,12aR)-2-methyl-6-(3-methylbenzo[b]thiophen-2-yl)-2,3,6,7,12,12a-hexahydropyrazino[1'2':1,6]-pyrido[3,4-b]indole-1,4-dione

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(1R,3R)-1-(3-Methylbenzo[b]thiophen-2-yl)-2,3,4,9-tetrahydro-1H- β -carboline-3-carboxylic acid methyl ester (1.75 g, 4.6 mmol) was treated as described below in Example 8 to provide 540 mg (28%) of Example 7.

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1H NMR (DMSO- d_6) δ : 11.21 (s, 1H), 7.82 (d, $J=8.05$ Hz, 2H), 7.54 (d, $J=7.68$ Hz, 1H), 7.46 (m, 6H), 7.21 (m, 2H), 7.18 (m, 2H), 4.51 (dd, $J=4.02, 11.7$ Hz, 1H), 4.27 (d, $J=17.9$ Hz, 1H), 4.07 (d, $J=17$ Hz, 1H), 3.34 (m, 2H), 3.06 (dd, $J=4.39, 19$, 1H), 2.90 (s, 3H), 2.54 (s, 3H). TOF MS ES+ extract mass calculated for $C_{24}H_{22}N_3O_2S$ (p+1): $m/z=416.1433$. Found: 416.1558.

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Preparation of Example 8

A solution of (1S,3R)-1-(3-methylbenzo[b]-thiophen-2-yl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (1.50 g. 3.90 mmol) in tetrahydrofuran (25 mL) was treated with triethylamine (2.5 mL). Chloroacetyl chloride (0.50 g, 4.4 mmol) was added and the solution was heated at reflux for 18 hours. A few additional drops of chloroacetyl chloride were added to drive the reaction to completion. After cooling, deionized water (10 mL) and ethyl acetate (200 mL) were added, the organic layer separated, washed once with dilute hydrochloric acid, dried (magnesium sulfate), filtered, and concentrated *in vacuo*. The residue was dissolved in tetrahydrofuran (50 mL) and treated with 2.5 M methyamine (1.0 mL) in tetrahydrofuran. The resulting solution was refluxed for 2 hours, cooled to room temperature, diluted with water, and extracted with ethyl acetate. The solution was dried (magnesium sulfate), filtered, and concentrated *in vacuo*.

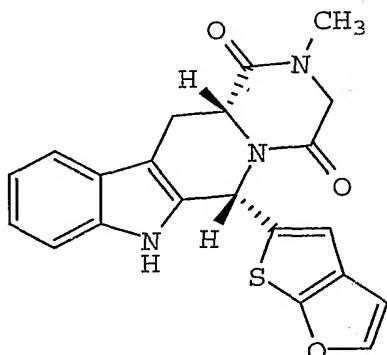
Chromatography (silica gel, 15% ethyl acetate/85% hexane to 40% ethyl acetate/60% hexane) of the residue provided 0.57 g (34%) of the title product as a yellow solid.

¹H NMR (DMSO-d₆) δ: 10.98 (s, 1H), 7.71 (m, 2H), 7.60 (d, J=6.95 Hz, 3H), 7.31 (m, 3H), 7.05 (m, 2H), 6.68 (s, 1H), 4.45 (dd, J=4.39, 11.71 Hz, 1H), 4.17 (d, J=18.66 Hz, 1H), 3.95 (d, J=17.56 Hz, 1H), 3.62 (dd, J=4.49, 15.73 Hz, 1H), 3.30 (d, J=7.31 Hz, 1H), 3.10 (m, 1H), 2.92 (s, 3H), 2.65 (s, 3H).

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Preparation of Example 9

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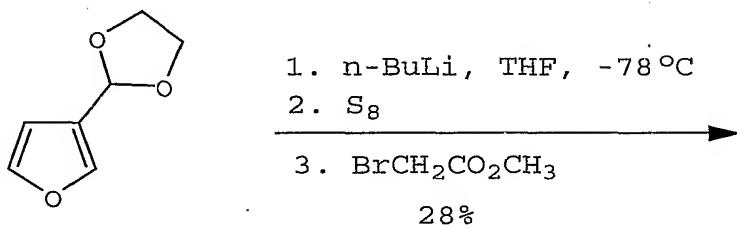


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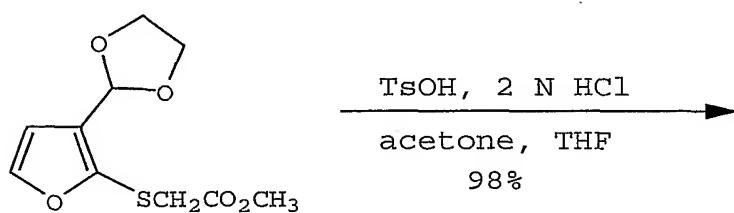
Example 9

Example 9 was prepared from D-tryptophan methyl ester hydrochloride and thieno[2,3-b]furan-5-carboxaldehyde (Intermediate 9) as depicted in the synthetic following scheme. Intermediate 9 was synthesized in five steps from a starting acetal according to known procedures. See G.D. Hartman et al., *J. Heterocyclic Chem.*, 27, 679 (1990).

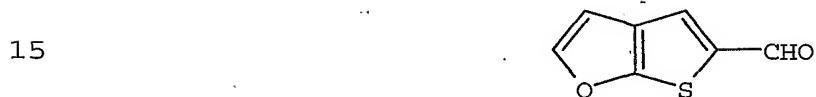
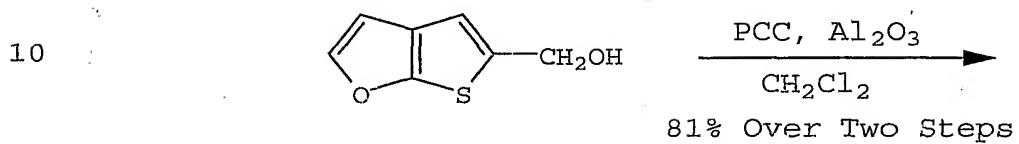
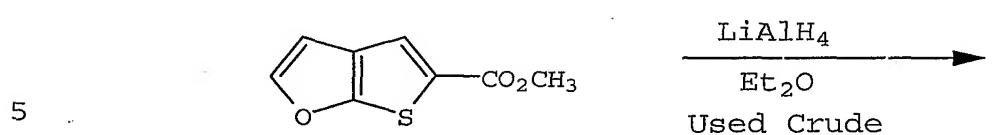
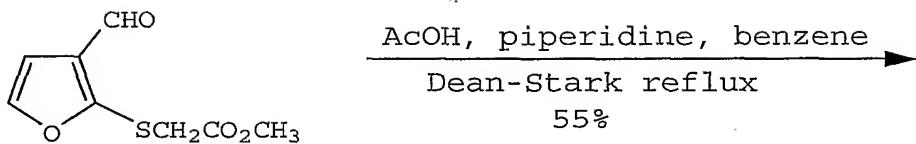
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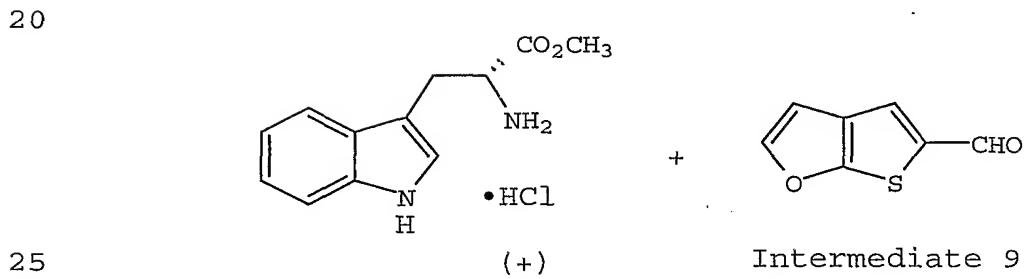
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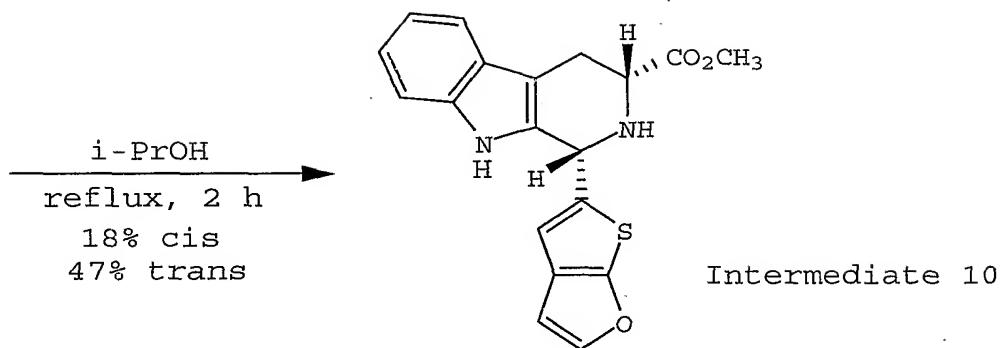
- 46 -



Intermediate 9



- 47 -



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$\xrightarrow[\text{Et}_3\text{N, CH}_2\text{Cl}_2]{\text{ClCOCH}_2\text{Cl}}$

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Intermediate 11

$\xrightarrow[\text{45}^\circ\text{C, 15 h}]{\text{MeNH}_2, \text{ THF, MeOH}}$

 61% Over Two Steps

Example 9

Preparation of Methyl 3-[2-(1,3-Dioxolanyl)]-furan-2-yl mercaptoacetate

5 To a mechanically stirred solution of starting acetal (27.6 g, 197 mmol) in anhydrous THF (100 mL) under a nitrogen blanket at -78°C was added n-butyllithium (126 mL, 201 mmol, 1.6 M solution in hexanes). The resulting light yellow slurry was stirred at -78°C for 45 minutes after which sulfur (6.7 g, 209 mmol) was added in three portions over 5 minutes. The resulting orange slurry was stirred at -78°C for 30 minutes, then at -50°C for 20 minutes to provide a deep purple suspension which again was cooled to -78°C. A solution of methyl bromoacetate (22.4 g, 240 mmol) in THF (20 mL) was added dropwise over 10 minutes to afford a cloudy yellow mixture. After stirring at -78°C for 30 minutes, the reaction mixture was allowed to gradually warm to -10°C over 1.5 hours and stirred at -10°C for an additional 20 minutes. The resulting brown, two-phase mixture was diluted with diethyl ether (500 mL), washed successively with brine (100 mL), water (2 x 75 mL), and brine (100 mL), dried over Na_2SO_4 , filtered and the solvent was removed under reduced pressure. The resulting residue was purified by flash column chromatography, eluting with hexanes/ethyl acetate (3:1), to provide the thioether as a yellow oil (11.4 g, 28%): TLC R_f (4:1 hexanes/ethyl acetate)=0.28; ^1H NMR (300 MHz, CDCl_3): δ : 7.48 (d, $J=2.0$ Hz, 1H), 6.51 (d, $J=2.0$ Hz, 1H), 5.87 (s, 1H), 4.15-3.90 (m, 4H), 3.69 (s, 3H), 3.50 (s, 2H) ppm.

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Preparation of (3-Formylfuran-2-yl)mercptoacetate

The above thioether (11.4 g, 47.0 mmol) and p-toluenesulfonic acid monohydrate (450 mg, 2.4 mmol) were stirred in a mixture of acetone (80 mL), THF (20 mL); and 2 N aqueous HCl (12 mL) at room temperature for 2 hours. The mixture was neutralized with a saturated NaHCO₃ solution, and the resulting suspension was concentrated to 20 mL under reduced pressure. The mixture was diluted with diethyl ether (300 mL), washed successively with water (30 mL), and brine (50 mL), dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure to provide the aldehyde as a brown oil which was used without further purification (9.24 g, 98%): TLC R_f (4:1 hexanes/ethyl acetate)=0.30; ¹H NMR (300 MHz, CDCl₃): δ: 10.0 (s, 1H); 7.51 (d, J=2.0 Hz, 1H), (d, J=2.0 Hz, 1H), s, 5H) ppm.

Preparation of Methyl Thieno[2,3-b]furan-5-carboxylate

Piperidine (5.60 mL, 57.3 mmol) was added to a solution of acetic acid (3.57 mL, 62.4 mmol) in benzene (200 mL) and the mixture was stirred at room temperature for 15 minutes. To this cloudy mixture was added a solution of the above aldehyde (9.24 g, 46.2 mmol) in benzene (50 mL). The resulting red solution was refluxed with water removal via a Dean-Stark trap for 4.5 hours under a nitrogen blanket. The resulting brown solution was cooled to room temperature and diluted with ether (250 mL). The organic solution was washed successively with water

- 50 -

(100 mL), 2 N aqueous HCl (30 mL), saturated NaHCO₃ solution, water (30 mL), and brine (50 mL), dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The residue was purified by 5 flash column chromatography, eluting with hexanes/- ethyl acetate (5:1), to provide the thienofuran as a pink solid (4.6 g, 55%): TLC R_f (4:1 hexanes/ethyl acetate)=0.79; ¹H NMR (300 MHz, CDCl₃): δ: 7.72 (s, 1H), 7.66 (d, J=2.2 Hz, 1H), 6.74 (d, J=2.2 Hz, 1H), 10 3.90 (s, 3H) ppm.

Preparation of Thieno[2,3-b]furan-5-carboxaldehyde (Intermediate 9)

15 To a suspension of lithium aluminum hydride (436 mg, 11.5 mmol) in ether (60 mL) at 0°C under a nitrogen blanket was added a solution of the above thienofuran (1.0 g, 5.49 mmol) in diethyl ether (20 mL) dropwise, after which the mixture was warmed to room temperature and stirred for 2 hours. The suspension was cooled to 0°C and treated successively with water (0.5 mL), 6 N aqueous sodium hydroxide (1.3 mL), and water (2.3 mL). The resulting salt was removed by filtration under reduced pressure, washed with ether (3 x 50 mL) and the filtrate was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to a volume of approximately 2 mL. This concentrated solution of thieno[2,3-20 b]furan-5-carbinol was diluted immediately with methylene chloride (50 mL) and used directly in the next step: TLC R_f (3:1 hexanes/ethyl acetate)=0.20.

To the above methylene chloride solution of the carbinol was added pyridinium chlorochromate

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(1.77 g, 8.63 mmol) and neutral aluminum oxide (4.0 g). The resulting mixture was stirred at room temperature under a nitrogen blanket for 1.5 hours. The black slurry was filtered through a short plug 5 of silica gel, eluting with diethyl ether (100 mL) followed by methylene chloride (100 mL). The filtrate was concentrated to afford Intermediate 9 as an oil which was used directly in the next step (674 mg, 81% over two steps): TLC R_f (3:1 hexanes/ethyl 10 acetate)=0.33; 1H NMR (300 MHz, $CDCl_3$): δ : 9.90 (s, 1H), 7.70 (d, $J=2.0$ Hz, 1H), 7.68 (s, 1H), 6.80 (d, $J=2.0$ Hz, 1H) ppm.

Preparation of cis- β -carboline (Intermediate 10)

15 A mixture of Intermediate 1 (674 mg, 4.43 mmol) and D-tryptophan methyl ester hydrochloride (1.18 g, 4.64 mmol) was refluxed in isopropyl alcohol (15 mL) under a nitrogen blanket for 2 hours. 20 The resulting yellow solution was cooled to room temperature and diluted with ethyl acetate (100 mL). The mixture was washed successively with saturated $NaHCO_3$ solution (15 mL), water (10 mL), and brine (10 mL), dried over Na_2SO_4 , filtered, and the solvent was 25 removed under reduced pressure. The residue was purified by flash column chromatography, eluting with hexanes/ethyl acetate (2:1), to provide Intermediate 10 as a white foam (285 mg, 18%): TLC R_f (2:1 hexanes/ethyl acetate)=0.33; 1H NMR (300 MHz, $DMSO-d_6$): δ : 7.7-7.50 (m, 3H), 7.30-7.09 (m, 3H), 7.08 (s, 1H), 6.67 (d, $J=2.4$ Hz, 1H), 5.55 (s, 1H), 3.92-4.00 (m, 1H), 3.83 (s, 3H), 3.27-3.17 (m, 1H), 3.08-2.93 (m, 1H) ppm.. The trans- β -carboline was

- 52 -

also obtained as a light yellow solid, but was not characterized (736 mg, 47%): TLC R_f (2:1 hexanes/-ethyl acetate)=0.22..

5 Preparation of cis-2-Chloroacetyl-
 β -carboline (Intermediate 11)

Chloroacetyl chloride (0.079 mL, 1.03 mmol) was added dropwise to a mixture of Intermediate 2 (280 mg, 0.80 mmol) and triethylamine (0.14 mL, 1.03 mmol) in methylene chloride (8 mL) at 0°C under a nitrogen blanket, and the resulting mixture was stirred at 0°C for 2 hours. The solution was diluted with ethyl acetate (60 mL), washed with a saturated $NaHCO_3$ solution (10 mL), and brine (10 mL), and the solvent was removed under reduced pressure to provide Intermediate 11 as a pink foam (405 mg), which was used without further purification or characterization.

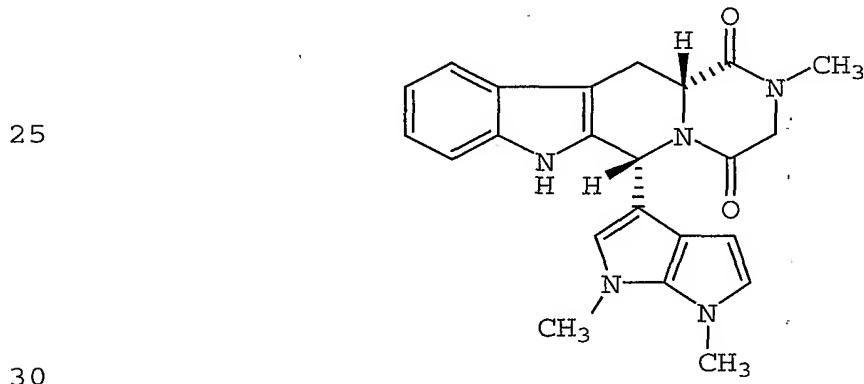
Preparation of Example 9

A mixture of the crude Intermediate 11 (405 mg) and methylamine (1.6 mL, 3.2 mmol, 2 M solution in THF) in methanol (7 mL) was heated at 45°C under a nitrogen blanket for 15 hours. The resulting solution was concentrated under reduced pressure to provide a yellow solid which was stirred in methanol (5 mL) for 1 hour. The solid was isolated by filtration under reduced pressure, washed with methanol (5 x 1 mL), and dried in a vacuum oven at 60°C for 17 hours to provide Example 9 as an off-white solid (189 mg, 61% over two steps): mp 275-

- 53 -

278°C; TLC R_f (5:1 methylene chloride/ethyl acetate)=0.22; ^1H NMR (500 MHz, DMSO- d_6): δ : 11.20 (s, 1H), 7.82 (d, $J=2.0$ Hz, 1H), 7.62 (d, $J=7.8$ Hz, 1H), 7.35 (d, $J=7.8$ Hz, 1H), 7.11 (t, $J=7.8$ Hz, 1H), 7.04 (t, $J=7.8$ Hz, 1H), 6.77 (s, 1H), 6.73 (d, $J=2.0$ Hz, 1H), 6.60 (s, 1H), 4.38 (dd, $J=11.7, 4.2$ Hz, 1H), 4.23 (d, $J=17.1$ Hz, 1H), 3.99 (d, $J=17.1$ Hz, 1H), 3.55 (dd, $J=15.8, 4.6$ Hz, 1H), 2.95-2.85 (m, 4H) ppm; ESI MS m/z 390 [$\text{C}_{21}\text{H}_{17}\text{N}_3\text{O}_3\text{S}-\text{H}$] $^+$; $[\alpha]_D^{25^\circ\text{C}}=+36.4$ (c=0.25, DMSO). Anal. Calcd. for $\text{C}_{21}\text{H}_{17}\text{N}_3\text{O}_3\text{S}$: C, 64.43; H, 4.38; N, 10.73. Found: C, 64.24; H, 4.39; N, 10.54. The stereochemistry of Example 9 was confirmed to be the desired cis isomer by a series of NOE difference experiments: a positive NOE enhancement from the C12a proton at 4.38 ppm to the C6 proton at 6.60 ppm; a positive NOE enhancement from the C6 proton at 6.60 ppm to the C12a proton at 4.38 ppm.

20 Example 10

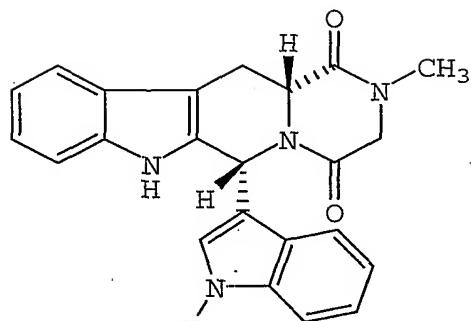


Example 10

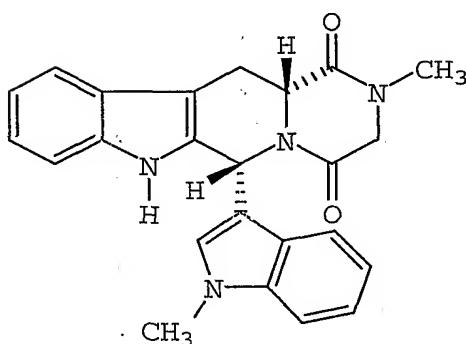
- 54 -

Example 11

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Example 12

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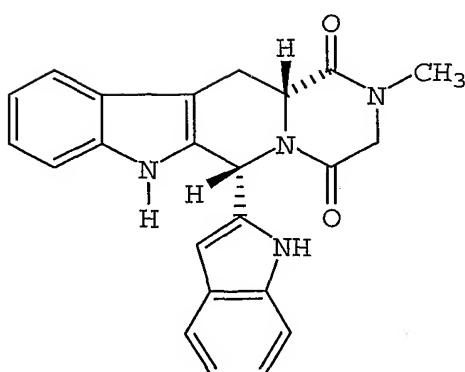
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Example 13

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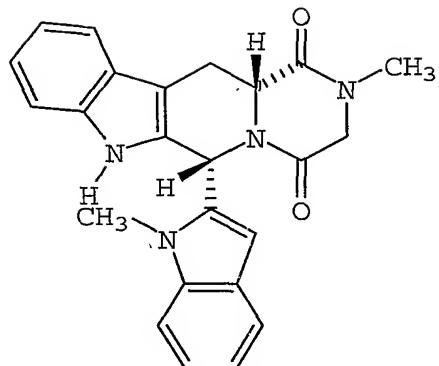
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Example 14

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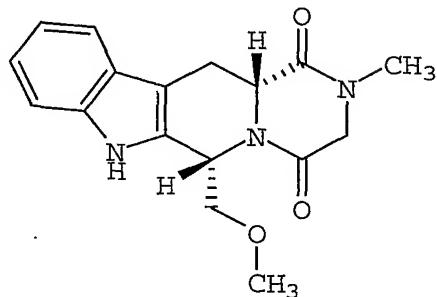


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Preparation of Example 15

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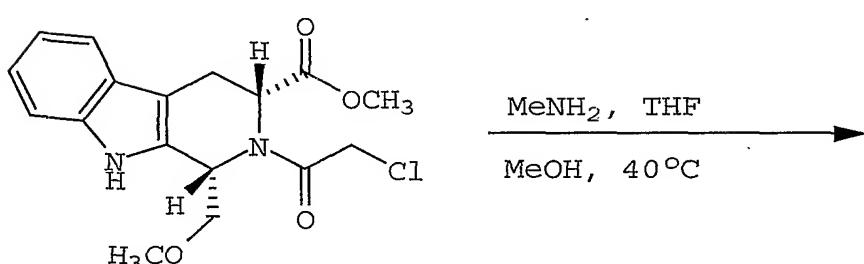
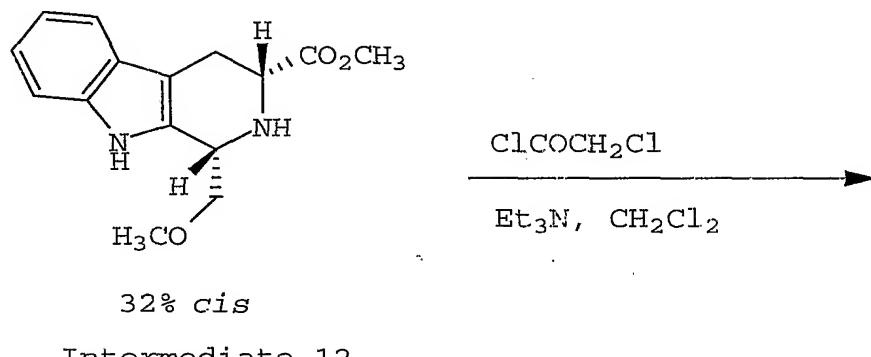
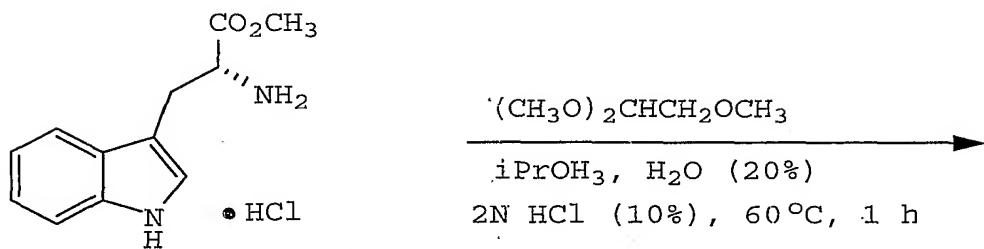
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Example 15 was prepared from a tryptophan ester of structural formula (IV), wherein R^0 is hydrogen. The tryptophan ester utilized to prepare

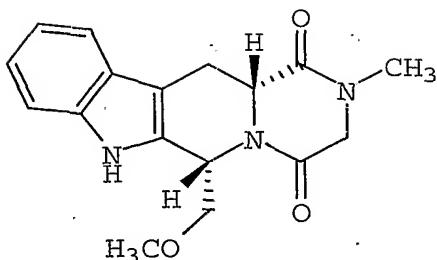
- 56 -

Example 15 is available commercially from Aldrich Chemical Co., Milwaukee, WI.



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66% for two steps

Example 15

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Intermediate 12Preparation of a (+)-cis-β-carboline

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A mixture of D-tryptophan methyl ester hydrochloride (1.27 g, 5.00 mmol), methoxyacetaldehyde dimethyl acetal (0.80 mL, 6.2 mmol) in isopropyl alcohol (12 mL) and water (2 mL) was stirred at 60°C under a nitrogen blanket for 20 minutes. To the resulting clear solution was added 2N HCl (1 mL), and the resulting yellow solution stirred at 60°C for 1 hour. The solution then was cooled to room temperature, diluted with ethyl acetate (100 mL), and neutralized with saturated aqueous sodium bicarbonate (NaHCO₃) (20 mL). The organic layer was washed with brine (20 mL), dried over sodium sulfate (Na₂SO₄), filtered, and the solvent was removed under reduced pressure to provide a yellow solid. The residue was purified by column chromatography, eluting with methylene chloride:ethyl acetate (4:1), to provide the (+)-cis carboline Intermediate 12 as a white solid (435 mg, 32%): TLC R_f (4:1 methylene

- 58 -

chloride/ethyl acetate)=0.28; ^1H NMR (300 MHz, CDCl_3): δ : 8.48 (bs, 1H), 7.50 (d, $J=8.0$ Hz, 1H), 7.34 (d, $J=8.0$ Hz, 1H), 7.20-7.03 (m, 2H), 4.42-4.34 (m, 1H), 3.87-3.54 (m, 6H), 3.50 (s, 3H), 3.20-3.10 (m, 1H), 2.90-2.76 (m, 1H). The *trans* carboline was incompletely eluted from the column: TLC R_f (4:1 methylene chloride/ethyl acetate)=0.19.

Intermediate 13

10 Preparation of a (+)-*cis*-2-Chloroacetyl- β -carboline

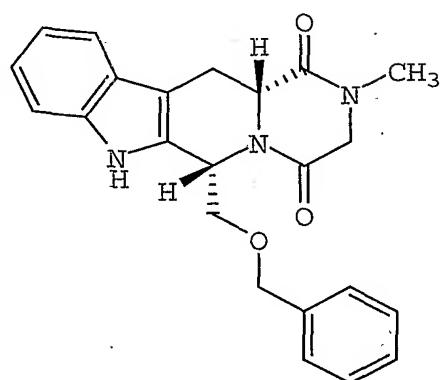
Chloroacetyl chloride (0.15 mL, 1.90 mmol) was added dropwise to a mixture of Intermediate 12 (435 mg, 1.59 mmol) and triethylamine (0.27 mL, 1.90 mmol) in methylene chloride (18 mL) at 0°C under a nitrogen blanket. The resulting mixture was stirred at 0°C for 2 hours, after which the mixture was diluted with methylene chloride (50 mL) and washed successively with water (15 mL) and brine (15 mL). The organic layer was concentrated under reduced pressure to yield Intermediate 13 as a yellow foam (590 mg), which was used without purification. TLC R_f (4:1 methylene chloride/ethyl acetate)=0.89.

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Preparation of Examples 16-18

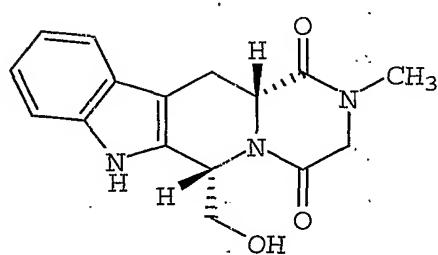
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Example 16

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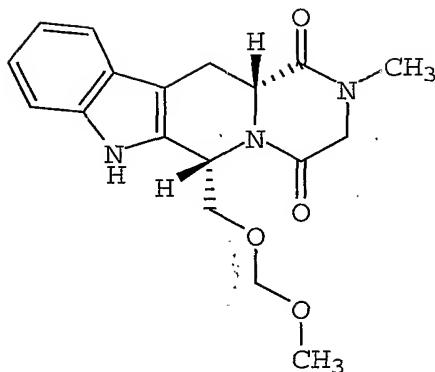
Example 17

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- 60 -

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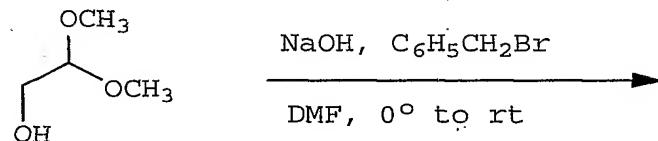


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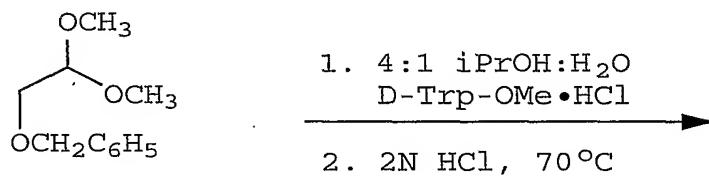
Example 18

Examples 16-18 were prepared starting with the identical D-tryptophan methyl ester (D-Trp-OMe·HCl) utilized in the preparation of Example 15, and with methoxyacetaldehyde dimethyl acetal by the following synthetic sequence.

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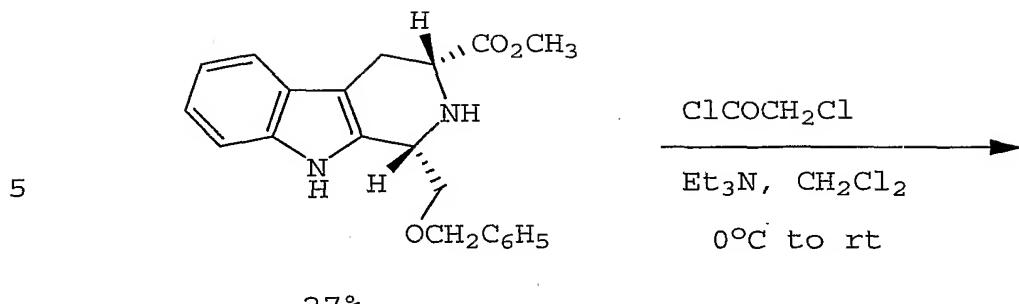


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Intermediate 14

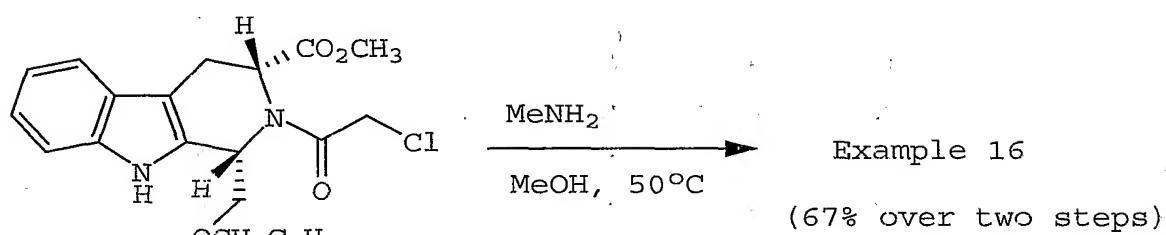
93%

- 61 -

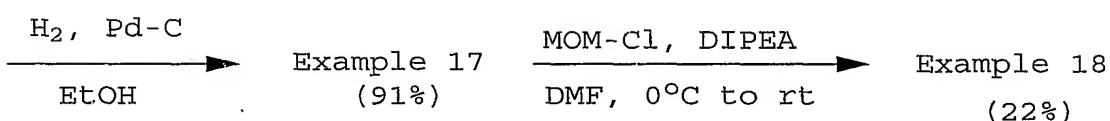


Intermediate 15

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Intermediate 16



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Intermediate 14

Preparation of a Dimethylacetal

A solution of methoxyacetaldehyde dimethylacetal (4.11 g, 38 mmol) and benzyl bromide (4.6 mL, 38 mmol) in N,N-dimethylformamide (17 mL) was cooled to 0°C. To this mixture was added sodium hydroxide pellets (1.66 g, 42 mmol), then the resulting mixture warmed slowly to room temperature overnight. The reaction mixture was poured into water and extracted with hexanes (2 x 200 mL). The combined organic extracts were washed with water, dried over Na₂SO₄, filtered, and concentrated under reduced pressure to yield Intermediate 14 as a pale yellow oil (7.03 g, 93%): ¹H NMR (300 MHz, CDCl₃): δ: 7.28 (s, 5H), 4.52 (s, 2H), 4.49 (t, J=5.2 Hz, 2H), 3.44 (d, J=5.1 Hz, 1H), 3.32 (s, 6H).

Intermediate 15

Preparation of a cis-β-Carboline

D-tryptophan methyl ester hydrochloride (4.07 g, 16 mmol) and Intermediate 14 (3.75 g, 19 mmol) were combined with stirring in a solution of IPA (80 mL) and water (20 mL). To the resulting mixture was added 2N HCl (2 mL) and the resulting mixture heated at 70°C for 18 hours. The mixture was neutralized with saturated aqueous NaHCO₃, then extracted with methylene chloride (2 x 400 mL). The combined organic extracts were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography, eluting with methylene chloride/acetone

- 63 -

(98:2), to provide the *cis*-carboline Intermediate 15 as an orange oil (1.5 g, .27%): TLC R_f (6:1 methylene chloride/ethyl acetate)=0.49; 1H NMR (300 MHz, $CDCl_3$): δ : 8.46 (s, 1H), 7.50 (d, $J=0.7$ Hz, 1H), 7.47-7.25 (m, 6H), 7.18-7.06 (m, 2H), 4.65 (s, 2H), 4.44-4.39 (m, 2H), 3.88-3.82 (m, 1H), 3.81 (s, 3H), 3.66 (t, $J=8.7$ Hz, 1H), 3.18-3.11 (m, 1H), 2.88-2.78 (m, 1H), 1.87 (bs, 1H).

10 Intermediate 16

Preparation of a *cis*-2-Chloroacetyl- β -carboline

Chloroacetyl chloride (0.30 mL, 3.7 mmol) was added dropwise to a mixture of Intermediate 15 (1.0 g, 2.9 mmol) and triethylamine (0.52 mL, 3.7 mmol) in methylene chloride (25 mL) at 0°C under an argon blanket. The resulting mixture was stirred at 0°C for 2 hours, after which the mixture was slowly warmed to room temperature overnight. The resulting yellow solution was diluted with methylene chloride, then washed successively with water, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 , filtered, and the solvent was removed under reduced pressure to provide Intermediate 16 as a tan foam which was used without purification (1.2 g): TLC R_f (6:1 methylene chloride/ethyl acetate)=0.94.

Example 16Preparation of (6S,12aR)-6-Benzylloxymethyl-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1'2':1,6]pyrido-[3,4-b]indole-1,4-dione

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A mixture of crude Intermediate 16 (about 1.2 g, 2.8 mmol), methylamine (7.0 mL, 2.0 M in THF, 13.5 mmol), and methanol (25 mL) was heated at 50°C under an argon blanket overnight. The resulting solution was cooled to room temperature. The solvent then was removed under reduced pressure to give an orange residue, which was purified by flash column chromatography, eluting with methylene chloride/ethyl acetate (5:1 to 2:1), to provide Example 11 as a pale yellow oil which crystallized under vacuum (0.83 g, 76%). A small sample was recrystallized from cold methylene chloride to give a white solid. The compound was confirmed to be the *cis*-isomer by NOE difference experiments (positive enhancement): mp 181-187°C; TLC R_f (4:1 methylene chloride/ethyl acetate)=0.29; ^1H NMR (300 MHz, DMSO- d_6): δ : 11.06 (s, 1H), 7.56 (d, $J=7.6$ Hz, 1H), 7.37 (d, $J=7.7$ Hz, 1H), 7.21-7.19 (m, 3H), 7.11-6.99 (m, 4H), 5.40 (t, $J=3.9$ Hz, 1H), 4.30 (s, 2H), 4.24-4.17 (m, 2H), 3.93 (d, $J=17.0$ Hz, 1H), 3.68 (d, $J=4.0$ Hz, 2H), 3.43-3.36 (m, 1H), 2.93 (s, 3H), 2.89-2.80 (m, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ : 167.0, 166.8, 138.1, 135.1, 132.0, 127.9, 127.2, 126.9, 125.7, 120.8, 118.6, 117.7, 111.2, 106.8, 71.9, 71.3, 54.8, 51.5, 51.3, 32.8, 22.3 ppm; CI MS (methane) m/z 268 ($\text{C}_{15}\text{H}_{13}\text{N}_3\text{O}_2+\text{H}^+$); $[\alpha]_D^{25^\circ\text{C}}=+45^\circ\text{C}$ ($c=0.5$, CHCl_3). Anal. Calcd. for $\text{C}_{23}\text{H}_{23}\text{N}_3\text{O}_3$: C, 70.93; H,

- 65 -

5.95; N, 10.79. Found: C, 70.54; H, 5.94; N, 10.60.

Example 17

5 Preparation of (6S,12aR)-6-Hydroxymethyl-2-
methyl-2,3,6,7,12,12a-hexahdropyrazino-
[1'2':1,6]pyrido[3,4-b]indole-1,4-dione

10 Example 16 (0.58 g, 1.5 mmol) was dissolved in ethanol (80 mL) and treated with a catalytic amount of 10% palladium on carbon (about 10% wet). The mixture was stirred under a hydrogen atmosphere at room temperature for 2.5 hours, after which the palladium catalyst was removed by vacuum filtration through a plug of Celite, eluting with methanol. The solvents were removed under reduced pressure to provide a yellow oil which was triturated with methanol/diethyl ether to provide Example 15 17 as a pale yellow solid (0.4 g, 91%). A small portion of the sample was recrystallized from methanol: mp 275-280°C; TLC R_f (4:1:0.5 methylene chloride/ethylacetate/methanol)=0.44; 1H NMR (300 MHz, DMSO- d_6): δ : 10.98 (s, 1H), 7.52 (d, $J=7.6$ Hz, 1H), 7.34 (d, $J=7.7$ Hz, 1H), 7.08-6.97 (m, 2H), 5.20 (t, $J=3.9$ Hz, 1H), 4.86 (t, $J=5.9$ Hz, 2H), 4.21-4.14 (m, 2H), 3.95 (d, $J=16.9$ Hz, 1H), 3.65-3.62 (m, 2H), 3.39-3.32 (m, 1H), 2.92 (s, 3H), 2.85-2.77 (m, 1H); 25 ^{13}C NMR (75 MHz, DMSO- d_6): δ : 167.0, 166.8, 135.9, 132.6, 125.8, 120.6, 118.5, 117.6, 111.2, 106.5, 63.2, 54.9, 53.8, 51.6, 32.8, 22.6 ppm; CI MS (methane) m/z 300 ($C_{16}H_{17}N_3O_3+H$) $^+$; $[\alpha]_D^{25^\circ C}=+131.1$ ($c=0.5$, methanol). Anal. Calcd. for $C_{16}H_{17}N_3O_3\cdot 0.25 H_2O$: C, 63.25; H, 5.81; N, 13.83. Found: C, 63.01; H, 5.73; N, 13.69.

Example 18

Preparation of (6S,12aR)-Methoxymethoxymethyl-2-methyl-2,3,6,7,12,12a-hexahdropyrazino-[1',2':1,6]pyrido[3,4-b]indole-1,4-dione

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A solution of Example 17 (0.2 g, 0.67 mmol) in N,N-dimethylformamide (10 mL) was cooled to 0°C. To this mixture was added successively diisopropylethylamine (DIPEA) (0.3 mL, 1.7 mmol) and chloromethyl methyl ether (MOM-Cl) (0.12 mL, 1.5 mmol). The resulting mixture was warmed slowly to room temperature overnight. The reaction mixture was poured into saturated aqueous NaHCO₃, and extracted with methylene chloride (3 x 100 mL). The combined organic extracts were washed with water and saturated aqueous ammonium chloride (NH₄Cl), dried over Na₂SO₄, filtered and concentrated under reduced pressure to yield Example 18 as a tan oil which solidified under vacuum. This residue was purified by flash column chromatography, eluting with methylene chloride/ethyl acetate/methanol (4:1:0.2), to provide Example 18 as a white solid (0.05 g, 22%). Recrystallization from cold methylene chloride/diethyl ether produced a white crystalline solid which was confirmed to be the *cis*-isomer by NOE difference experiments (positive enhancement): mp 180-185°C; TLC R_f (4:1:0.2 methylene chloride/ethyl acetate/methanol)=0.43. ¹H NMR (300 MHz, Benzene-d₆): δ: 7.53 (d, J=7.4 Hz, 1H), 7.49 (s, 1H), 7.24-7.12 (m, 3H), 5.39-5.36 (m, 1H), 4.27-4.22 (m, 2H), 4.00-3.95 (m, 1H), 3.78-3.71 (m, 1H), 3.64-3.59 (m, 1H), 3.38-3.08 (m, 4H), 2.86 (s, 2H), 2.38 (s, 3H); ¹³C NMR (75 MHz, Benzene-d₆): δ: 167.1, 166.6, 136.9,

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132.2, 122.7, 120.5, 119.7, 111.7, 108.5, 96.8,
69.9, 56.1, 55.1, 52.7, 52.4, 33.1, 30.5, 24.6 ppm;
CI MS (methane) m/z 344 ($C_{18}H_{21}N_3O_4 + H$) $^+$; $[\alpha]_D^{25^\circ C} = +40.9$
($c=0.32$, benzene-d₆). Anal. Calcd. for $C_{18}H_{21}N_3O_4$: C,
5 H, 6.16; N, 12.24. Found: C, 62.94; H,
6.12; N, 12.14.

The following are additional examples of
compounds of structural formula (I) that can be
prepared by methods analogous to the preparation of
10 Examples 1-18.

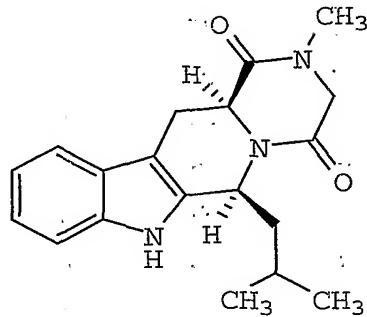
Example 19

(+,-,cis)-6-Isobutyl-2-methyl-2,3,6,7,12,12a-hexa-
hydropyrazino[1'2':1,6]pyrido[3,4-b]indole-1,4-dione

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Example 20

(+,-, cis) -6-Cyclohexyl-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1'2':1,6]pyrido[3,4-b]indole, 1,4-dione

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Example 21

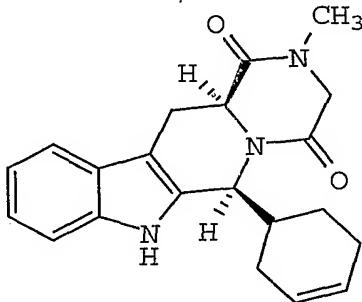
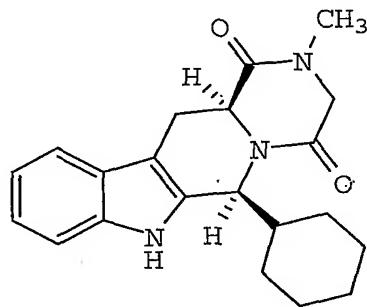
(+,-, cis) -6-Cyclohex-3-enyl-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1'2':1,6]pyrido[3,4-b]indole-1,4-dione

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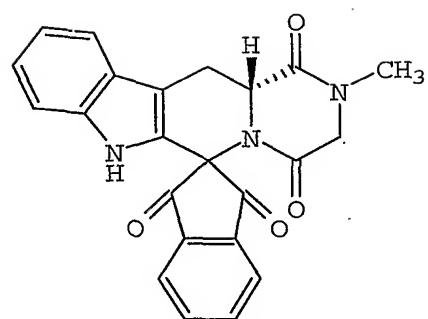
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Example 22

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Example 23

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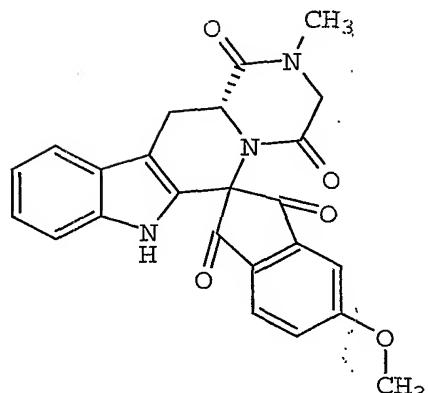
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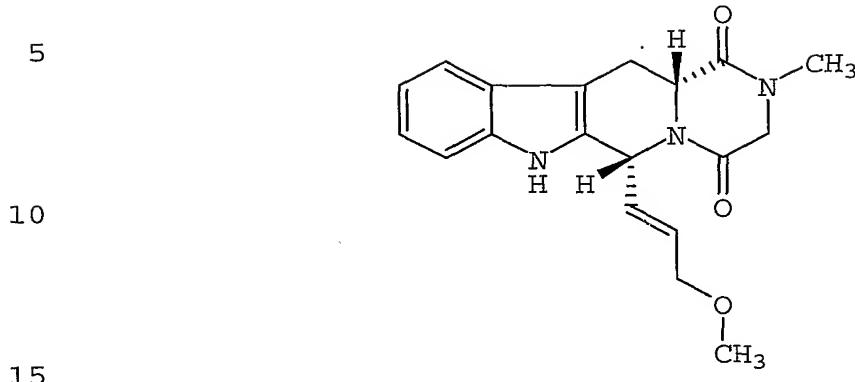
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Example 24



Compounds of the present invention can be formulated into tablets for oral administration.

For example, a compound of formula (I) can be formed 20 into a dispersion with a polymeric carrier by the coprecipitation method set forth in WO 96/38131, incorporated herein by reference. The coprecipitated dispersion then can be blended with excipients, then pressed into tablets, which optionally 25 are film-coated.

The compounds of structural formula (I) were tested for an ability to inhibit PDE5. The ability of a compound to inhibit PDE5 activity is related to the IC_{50} value for the compound, i.e., the 30 concentration of inhibitor required for 50% inhibition of enzyme activity. The IC_{50} value for compounds of structural formula (I) were determined using recombinant human PDE5.

The compounds of the present invention typically exhibit an IC_{50} value against recombinant 35 human PDE5 of less than about 50 μ M, and preferably less than about 25 μ M, and more preferably less than about 15 μ M. The compounds of the present invention

- 71 -

typically exhibit an IC_{50} value against recombinant human PDE5 of less than about 1 μM , and often less than about 0.05 μM . To achieve the full advantage of the present invention, a present PDE5 inhibitor 5 has an IC_{50} of about 0.1 nM to about 15 μM .

The production of recombinant human PDEs and the IC_{50} determinations can be accomplished by well-known methods in the art. Exemplary methods are described as follows:

10

EXPRESSION OF HUMAN PDEs

Expression in *Saccharomyces cerevisiae* (Yeast)

15 Recombinant production of human PDE1B, PDE2, PDE4A, PDE4B, PDE4C, PDE4D, PDE5, and PDE7 was carried out similarly to that described in Example 7 of U.S. Patent No. 5,702,936, incorporated herein by reference, except that the yeast transformation 20 vector employed, which is derived from the basic ADH2 plasmid described in Price et al., *Methods in Enzymology*, 185, pp. 308-318 (1990), incorporated yeast ADH2 promoter and terminator sequences and the *Saccharomyces cerevisiae* host was the protease-deficient strain BJ2-54 deposited on August 31, 1998 25 with the American Type Culture Collection, Manassas, Virginia, under accession number ATCC 74465. Transformed host cells were grown in 2X SC-leu medium, pH 6.2, with trace metals, and vitamins. After 24 30 hours, YEP medium-containing glycerol was added to a final concentration of 2X YET/3% glycerol. Approximately 24 hr later, cells were harvested, washed, and stored at -70°C.

HUMAN PHOSPHODIESTERASE PREPARATIONSPhosphodiesterase Activity Determinations

5 Phosphodiesterase activity of the preparations was determined as follows. PDE assays utilizing a charcoal separation technique were performed essentially as described in Loughney et al. (1996). In this assay, PDE activity converts [³²P]cAMP or

10 [³²P]cGMP to the corresponding [³²P]5'-AMP or [³²P]5'-GMP in proportion to the amount of PDE activity present. The [³²P]5'-AMP or [³²P]5'-GMP then was quantitatively converted to free [³²P]phosphate and unlabeled adenosine or guanosine by the action

15 of snake venom 5'-nucleotidase. Hence, the amount of [³²P]phosphate liberated is proportional to enzyme activity. The assay was performed at 30°C in a 100 μ L reaction mixture containing (final concentrations) 40 mM Tris HCl (pH 8.0), 1 μ M ZnSO₄, 5 mM

20 MgCl₂, and 0.1 mg/mL bovine serum albumin (BSA). PDE enzyme was present in quantities that yield <30% total hydrolysis of substrate (linear assay conditions). The assay was initiated by addition of substrate (1 mM [³²P]cAMP or cGMP), and the mixture

25 was incubated for 12 minutes. Seventy-five (75) μ g of Crotalus atrox venom then was added; and the incubation was continued for 3 minutes (15 minutes total). The reaction was stopped by addition of 200 μ L of activated charcoal (25 mg/mL suspension in 0.1 M NaH₂PO₄, pH 4). After centrifugation (750 X g for 3 minutes) to sediment the charcoal, a sample of the supernatant was taken for radioactivity determina-

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tion in a scintillation counter and the PDE activity was calculated.

Purification of PDE5 from *S. cerevisiae*

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Cell pellets (2.9 g) were thawed on ice with an equal volume of Lysis Buffer (25 mM Tris HCl, pH 8, 5 mM MgCl₂, 0.25 mM DTT, 1 mM benzamidine, and 10 μ M ZnSO₄). Cells were lysed in a Microfluidizer[®] (Microfluidics Corp.) using nitrogen at 20,000 psi. The lysate was centrifuged and filtered through 0.45 μ m disposable filters. The filtrate was applied to a 150 mL column of Q SEPHAROSE[®] Fast-Flow (Pharmacia). The column was washed with 1.5 volumes of Buffer A (20 mM Bis-Tris Propane, pH 6.8, 1 mM MgCl₂, 0.25 mM DTT, 10 μ M ZnSO₄) and eluted with a step gradient of 125 mM NaCl in Buffer A followed by a linear gradient of 125-1000 mM NaCl in Buffer A. Active fractions from the linear gradient were applied to a 180 mL hydroxyapatite column in Buffer B (20 mM Bis-Tris Propane (pH 6.8), 1 mM MgCl₂, 0.25 mM DTT, 10 μ M ZnSO₄, and 250 mM KCl). After loading, the column was washed with 2 volumes of Buffer B and eluted with a linear gradient of 0-125 mM potassium phosphate in Buffer B. Active fractions were pooled, precipitated with 60% ammonium sulfate, and resuspended in Buffer C (20 mM Bis-Tris Propane, pH 6.8, 125 mM NaCl, 0.5 mM DTT, and 10 μ M ZnSO₄). The pool was applied to a 140 mL column of SEPHACRYL[®] S-300 HR and eluted with Buffer C. Active fractions were diluted to 50% glycerol and stored at -20°C.

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The resultant preparations were about 85% pure by SDS-PAGE. These preparations had specific activities of about 3 μ mol cGMP hydrolyzed per minute per milligram protein.

5

Inhibitory Effect on cGMP-PDE

cGMP-PDE activity of compounds of the present invention was measured using a one-step assay adapted from Wells et al., *Biochim. Biophys. Acta*, 384, 430 (1975). The reaction medium contained 50 mM Tris-HCl, pH 7.5, 5 mM magnesium acetate, 250 μ g/ml 5'-Nucleotidase, 1 mM EGTA, and 0.15 μ M 8-[H^3]-cGMP. Unless otherwise indicated, the enzyme used was a human recombinant PDE5 (ICOS Corp., Bothell, Washington).

Compounds of the invention were dissolved in DMSO finally present at 2% in the assay. The incubation time was 30 minutes during which the total substrate conversion did not exceed 30%.

The IC₅₀ values for the compounds examined were determined from concentration-response curves typically using concentrations ranging from 10 nM to 10 μ M. Tests against other PDE enzymes using standard methodology showed that compounds of the invention are selective for the cGMP-specific PDE enzyme.

Biological Data

30 The compounds according to the present invention were typically found to exhibit an IC₅₀ value of less than 500 nM. An *in vitro* test data

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for representative compounds of the invention is given in the following table:

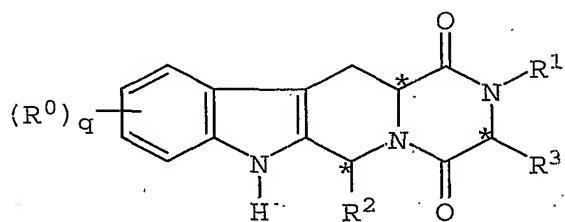
Table 1. *In vitro* results

Example	PDE5 IC ₅₀ (nM)
1	130
2	8726
3	16
4	880
5	25
6	40
7	1570
8	1
9	12
15	333
16	1,500
17	23,000
18	195
19	4,800
20	230 (bovine aorta)
21	560

Obviously, many modifications and variations of the invention as hereinbefore set forth can be made without departing from the spirit and scope thereof, and, therefore, only such limitations should be imposed as are indicated by the appended claims.

WHAT IS CLAIMED IS:

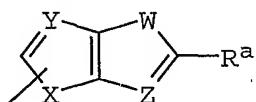
1. A compound having a formula



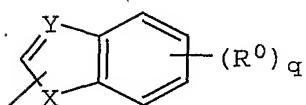
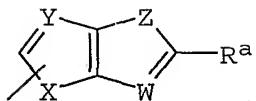
wherein R^0 , independently, is selected from the group consisting of halo and C_{1-6} alkyl;

R^1 is selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, $haloC_{1-6}$ alkyl, C_{3-8} cycloalkyl, C_{3-8} cycloalkyl C_{1-3} alkyl, aryl C_{1-3} alkyl, and heteroaryl C_{1-3} alkyl;

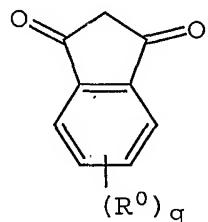
R^2 is selected from the group consisting of hydrogen, C_{1-6} alkyl, aryl C_{1-3} alkyl, C_{1-3} alkenylaryl, $haloC_{1-6}$ alkyl, C_{1-4} alkylene $C(=O)OR^a$, C_{1-4} alkylene $C(=O)NR^aR^b$, C_{3-8} cycloalkyl, Het, C_{3-8} cycloalkenyl, C_{3-8} heterocycloalkenyl, C_{1-4} alkyleneHet, C_{1-4} alkyleneQR^a, C_{2-6} alkenyleneQR^a, C_{1-4} alkyleneQC₁₋₄alkyleneQR^a,



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and a spiro substituent having the structure



R³ is selected from the group consisting of hydrogen and C₁₋₆alkyl,

or R¹ and R³ together represent a 3- or 4-membered alkyl or alkenyl chain component of a 5- or 6-membered ring;

R^a and R^b, independently, are selected from the group consisting of hydrogen, C₁₋₆alkyl, aryl, heteroaryl, arylC₁₋₃alkyl, heteroarylC₁₋₃alkyl, C₁₋₃alkylenearyl, C₁₋₃alkyleneheteroaryl, and Het;

R^c is null or is selected from the group consisting of hydrogen, C₁₋₆alkyl, aryl, heteroaryl, arylC₁₋₃alkyl, heteroarylC₁₋₃alkyl, C₁₋₃alkylenearyl, C₁₋₃alkyleneheteroaryl, and Het;

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Het is a 5- or 6-membered heterocyclic group, saturated or partially unsaturated, containing at least one heteroatom selected from the group consisting of oxygen, nitrogen, and sulfur, and optionally substituted with C_{1-4} alkyl or $C(=O)OR^a$;

Q is O, S, or NR^a ;

W is O, S, or NR^c ;

X is O, S, or NR^a ;

Y is CR^a or N;

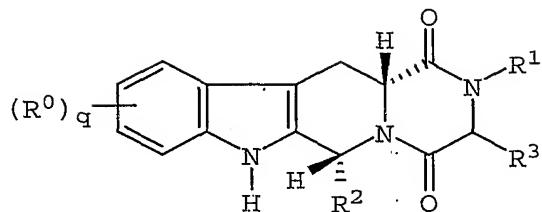
Z is CR^a , $C(R^a)_2$, or NR^a ; and

q is 0, 1, 2, 3, or 4,

and pharmaceutically acceptable salts and solvates thereof.

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2. The compound of claim 1 represented by the formula



and pharmaceutically acceptable salts and solvates thereof.

3. The compound of claim 1 wherein R⁰ is hydrogen.

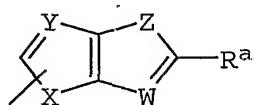
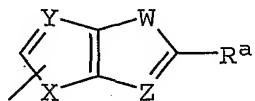
4. The compound of claim 1 wherein R¹ is selected from the group consisting of hydrogen, C₁₋₄alkyl, haloC₁₋₄alkyl, optionally substituted benzyl, C₃₋₆cycloalkylmethyl, pyridylC₁₋₃alkyl, and furylC₁₋₃alkyl.

5. The compound of claim 1 wherein R^3 is hydrogen.

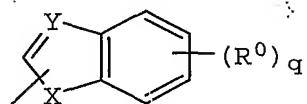
6. The compound of claim 1 wherein R¹ and R³ together represent a 3- or 4-membered alkyl chain component.

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7. The compound of claim 1 wherein R² is selected from the group consisting of hydrogen, C₁₋₆alkyl, arylC₁₋₃alkyl, C₁₋₃alkenylaryl, C₃₋₆cycloalkyl, Het, C₃₋₆cycloalkenyl, C₁₋₄alkyleneHet, C₁₋₄alkyleneQR^a, C₂₋₄alkenyleneQR^a, C₁₋₄alkyleneQC₁₋₄-alkyleneQR^a,

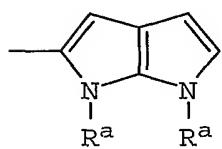
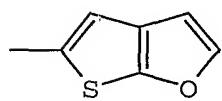
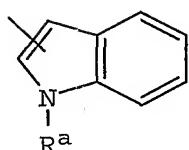
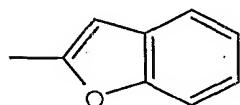
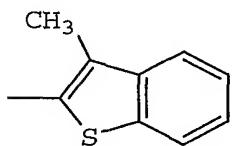


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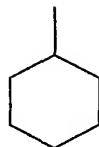
- 81 -

8. The compound of claim 1 wherein R² is selected from the group consisting of

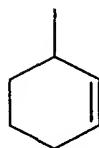


- 82 -

-CH₂OR^a, -CH₂OCH₂OR^a, -CH=CHCH₂OR^a,



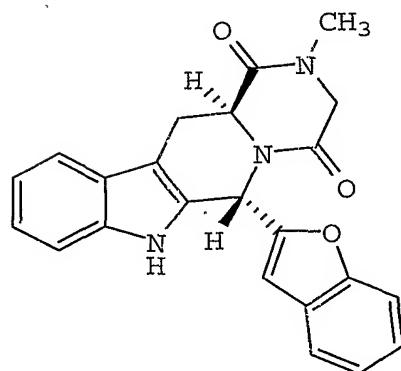
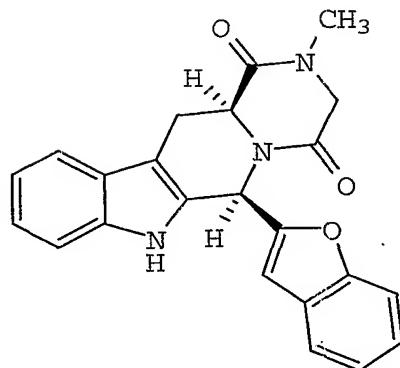
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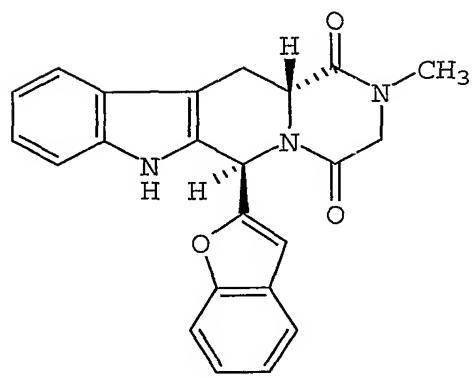
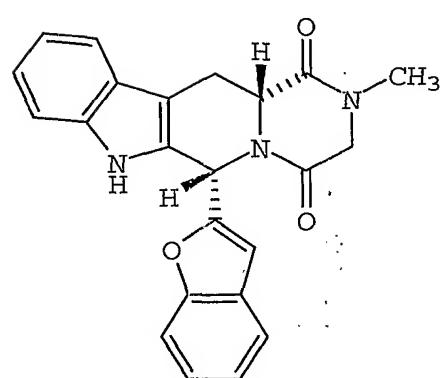
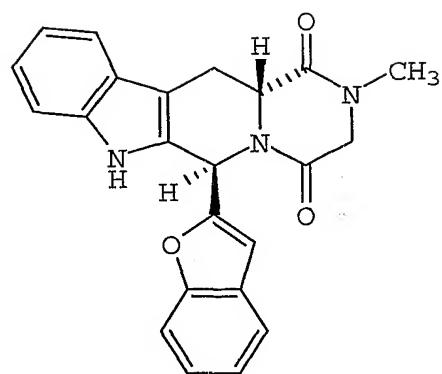
9. The compound of claim 8 wherein R^a is selected from the group consisting of hydrogen, C₁₋₆alkyl, and benzyl.

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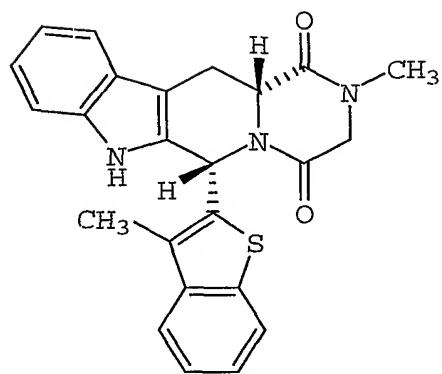
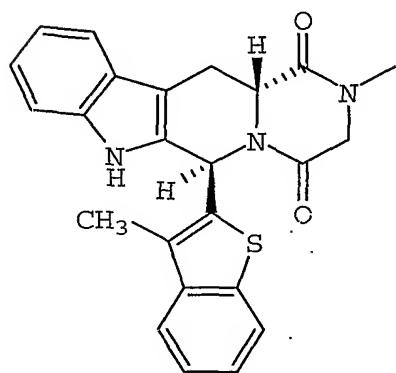
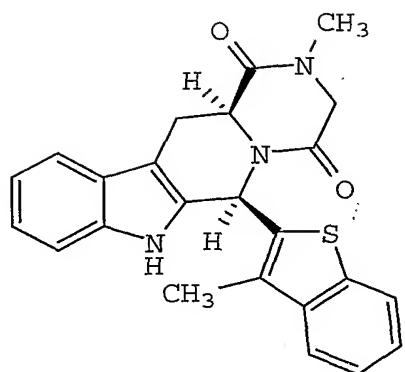
10. A compound selected from the group consisting of



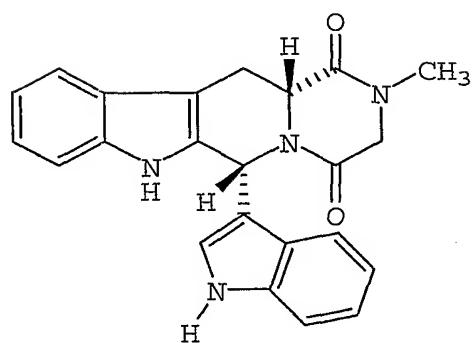
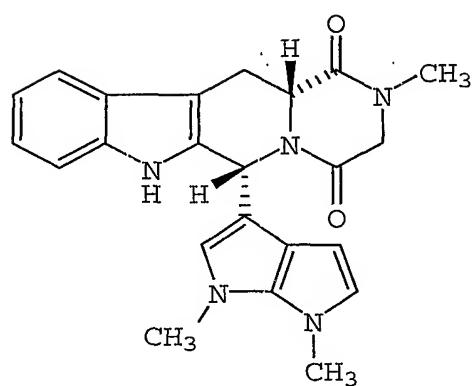
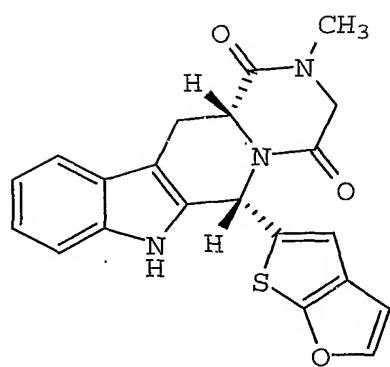
- 84 -



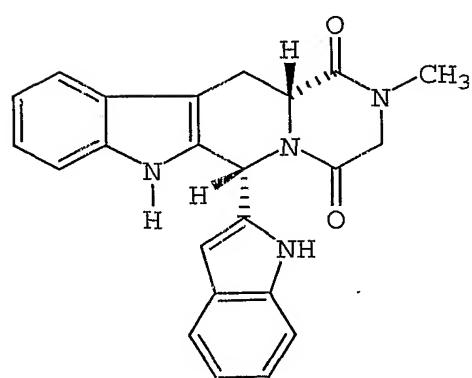
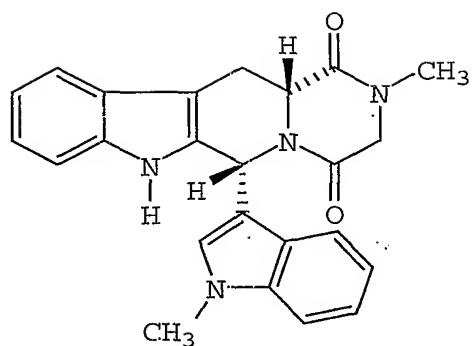
- 85 -



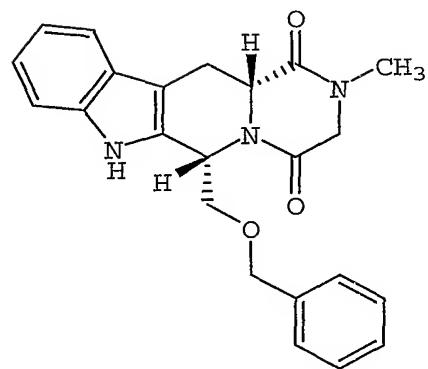
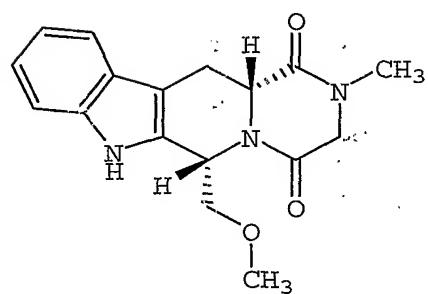
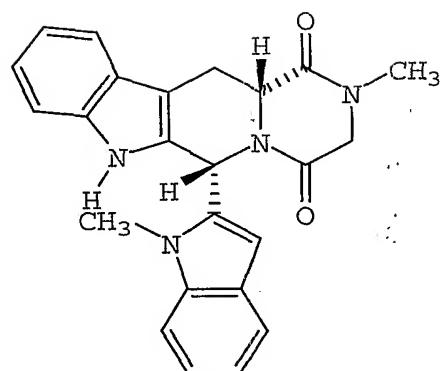
- 86 -



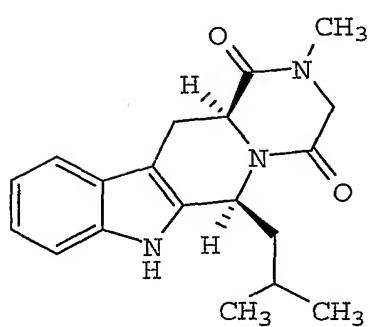
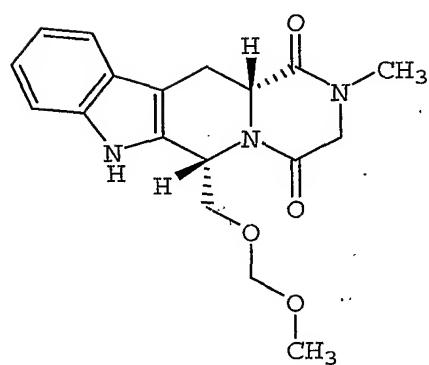
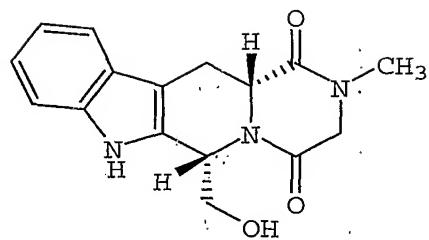
- 87 -



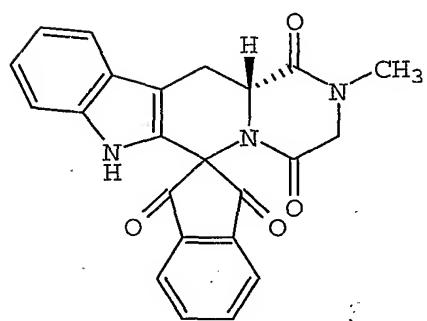
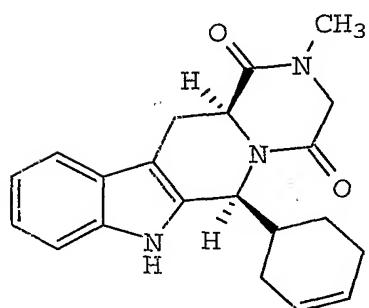
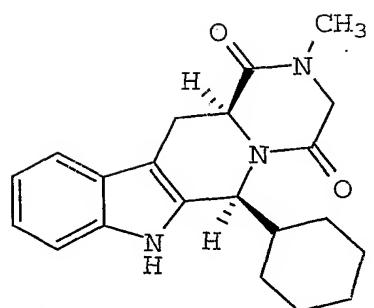
- 88 -



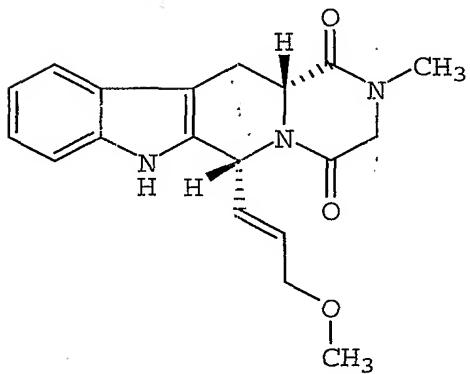
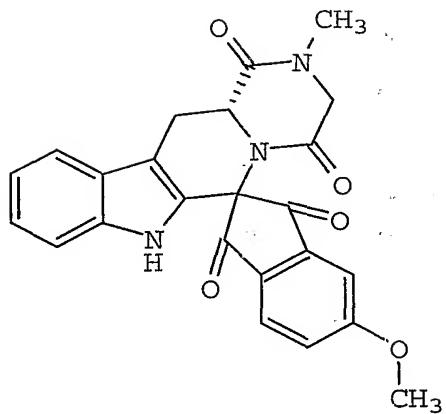
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and pharmaceutically acceptable salts and solvates thereof.

11. A pharmaceutical composition comprising a compound of claim 1, together with a pharmaceutically acceptable diluent or carrier.

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12. A method of treating a male or female animal for a condition where inhibition of a cGMP-specific PDE is of a therapeutic benefit comprising administering to said animal an effective amount of a pharmaceutical composition comprising a compound of claim 1, together with a pharmaceutically acceptable diluent or carrier.

13. The method of claim 12 wherein the condition is male erectile dysfunction.

14. The method of claim 13 wherein the treatment is an oral treatment.

15. The method of claim 12 wherein the condition is female arousal disorder.

16. The method of claim 15 wherein the treatment is an oral treatment.

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17. The method of claim 12 wherein the condition is selected from the group consisting of stable angina, unstable angina, variant angina, hypertension, pulmonary hypertension, chronic obstructive pulmonary disease, malignant hypertension, pheochromocytoma, acute respiratory distress syndrome, congestive heart failure, acute renal failure, chronic renal failure, atherosclerosis, a condition of reduced blood vessel patency, a peripheral vascular disease, a vascular disorder, thrombocythemia, an inflammatory disease, myocardial infarction, stroke, bronchitis, chronic asthma, allergic asthma, allergic rhinitis, glaucoma, peptic ulcer, a gut motility disorder, postpercutaneous transluminal coronary angioplasty, carotid angioplasty, post-bypass surgery graft stenosis, osteoporosis, preterm labor, benign prostatic hypertrophy, and irritable bowel syndrome.

18. A method of treating a condition where inhibition of a cGMP-specific PDE is of therapeutic benefit, in a human or a nonhuman animal body, comprising administering to said body a therapeutically effective amount of a compound of claim 1.

19. A method for the curative or prophylactic treatment of male erectile dysfunction or female arousal disorder, comprising administration of an effective dose of a compound of claim 1, and pharmaceutically acceptable salts and solvates thereof, to an animal.

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20. Use of a compound of claim 1 for the manufacture of a medicament for the curative or prophylactic treatment of a condition where inhibition of a cGMP-specific PDE is of a therapeutic benefit.

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11 April 2002 (11.04.2002)

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(10) International Publication Number
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[US/US]; 16110 N.E. 175th Street, Woodinville, WA 98072 (US).

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(74) Agent: NAPOLI, James, J.; Marshall, Gerstein & Borun, 6300 Sears Tower, 233 South Wacker Drive, Chicago, IL 60606 (US).

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(71) Applicant (for all designated States except US): LILLY ICOS LLC [US/US]; 1209 Orange Street, Wilmington, DE 19801 (US).

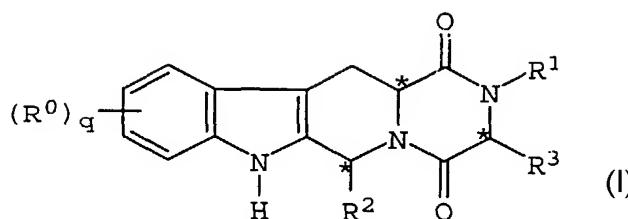
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(72) Inventors; and

(75) Inventors/Applicants (for US only): ORME, Mark, W. [US/US]; 4235 Francis Avenue, #203, Seattle, WA 98103 (US). SAWYER, Jason, Scott [US/US]; 5718 North Winthrop Avenue, Indianapolis, IN 46220 (US). DAUGAN, Alain, Claude-Marie [FR/FR]; Centre de Recherche Glaxo Smith Kline Courtaboeuf Z.A. de Courtaboeuf, 25, avenue de Québec, F-91940 Les Ulis (FR). BROWN, Raymond, F. [US/US]; 10518 Greenway Drive, Fishers, IN 46038 (US). SCHULTZE, Lisa, M.

WO 02/028858 A3

(54) Title: HEXAHYDROPYRAZINO '1'2': 1, 6!- PYRIDO '3, 4-B! INDOLE-1, 4-DIONE DERIVATIVES FOR THE TREATMENT OF CARDIOVASCULAR DISORDERS AND ERECTILE DISFUNCTION



(57) Abstract: Compounds of the general structural formula (I), and use of the compounds and salts and solvates thereof, as therapeutic agents.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/28972

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D471/14 C07D519/00 C07D471/20 A61K31/5025 A61K31/503
 A61P9/00 //((C07D471/14, 241:00, 221:00, 209:00), (C07D519/00,
 495:04, 471:14), (C07D519/00, 471:14, 471:04), (C07D471/20, 241:00,

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category ^a	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HE, HAIYIN ET AL: "Fumitremorgin C analog that reverse mitoxantrone resistance in human colon carcinoma cells" MEDICINAL CHEMISTRY RESEARCH (1999), 9(6), 424-437, XP000107366 page 424, abstract and page 427, table 2, compounds 10-22 and 25 ---	1,11-13
X	WO 95 19978 A (GLAXO) 27 July 1995 (1995-07-27) page 1, paragraph 1; claims 1,11,17; examples 51-60 --- -/-	1,11,12

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Date of the actual completion of the international search

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31/05/2002

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European Patent Office, P.B. 5818 Patentlaan 2
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INTERNATIONAL SEARCH REPORT

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PCT/US 01/28972A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 221:00, 209:00

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>MADRIGAL A ET AL: "The fate of the tryptophan stereocenter in the synthesis of 7,10,16,16a-tetrahydro-11H-quinazolino'2', 3':3,4:pyrazino'1,2-b!beta-carboline-5,8-diones"</p> <p>TETRAHEDRON: ASYMMETRY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 11, no. 17, 8 September 2000 (2000-09-08), pages 3515-3526, XP004224131</p> <p>pages 3523-24, compounds 9,10</p> <p>---</p> <p>-/-</p>	1

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Date of the actual completion of the international search

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Authorized officer

Alfaro Faus, I

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/28972

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WANG H ET AL: "THE N-ACYLIMINIUM PICTET-SPENGLER CONDENSATION AS A MULTICOMPONENT COMBINATORIAL REACTION ON SOLID PHASE AND ITS APPLICATION TO THE SYNTHESIS OF DEMETHOXYFUMITREMORGIN C ANALOGUES" ORGANIC LETTERS, ACS, WASHINGTON, DC, US, vol. 1, no. 10, 1999, pages 1647-1649, XP001021212 table 1, compounds 2 and 3 ---	1
X	SCOTT EDMONDSON ET AL.: "Total Synthesis of spirotryprostatin A, leading to the discovery of some biologically promising analogues" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 121, no. 10, 1999, pages 2147-2155, XP002198414 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863 page 2152, column 1, compound 20 and deprotection of 20 ---	1
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A	WO 97 03985 A (GLAXO WELLCOME) 6 February 1997 (1997-02-06) claims 1,9 ---	1,18
A	WO 97 03675 A (GLAXO WELLCOME) 6 February 1997 (1997-02-06) claims 1,3 ---	1,19
		-/-

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/28972

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PANDEY S K ET AL: "Microwave assisted stereospecific synthesis of (S)-3-substituted 2,3,6,7,12,12a-hexahydropyrazino'1',2':1,6'!pyrido'3,4-b!indole-1,4-dione" TETRAHEDRON, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 57, no. 20, 14 May 2001 (2001-05-14), pages 4437-4442, XP004239470 compounds 7a, 7b, 7g -----	1
E	WO 02 11706 A (NICOX) 14 February 2002 (2002-02-14) page 19, lines 3-5, compound C13 -----	1,11-13

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Information on patent family members

International Application No

PCT/US 01/28972

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/28972

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WO 0211706	A	14-02-2002	IT MI20001847 A1 WO 0211706 A2	08-02-2002 14-02-2002